"PHARMACOGNOSTICAL, PHYTOCHEMICAL AND PHARMACOLOGICAL STUDIES ON LEAVES OF *CARDIOSPERMUM HELICACABUM, LINN.*"

DISSERTATION

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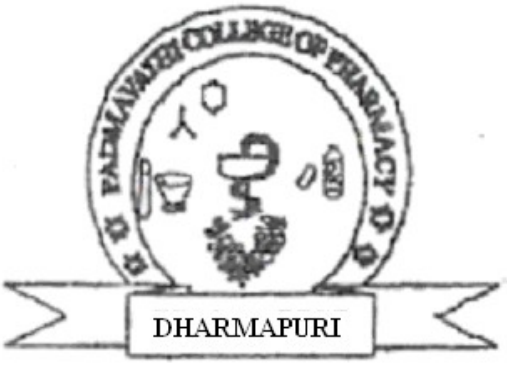

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CERTIFICATE

This is to certify that the dissertation entitled "**Pharmacognostical, Phytochemical and Pharmacological studies on leaves of *Cardiospermum helicacabum, Linn***." constitutes the original work carried out by Mr.RAJKUMAR MALLAPPA GADADE, B.Pharm. under the guidance and supervision of. Asst.Prof. M. RAJKUMAR, M.Pharm. Department of Pharmacognosy, Padmavathi College of Pharmacy, Periyanahalli, Dharmapuri-635205.

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DEDICATED TO MY

BELOVED

MOTHER

FATHER

FAMILY MEMBERS

TEACHERS

FRIENDS
AND
ALMIGHTY

ACKNOWLEDGEMENT

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Date:
Rajkumar Mallappa Gadade.

Place:

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1. INTRODUCTION

1.1 PREFACE

Nature always stands as a golden mark to exemplify the out standing phenomenon of symbiosis. The biotic and abiotic elements are all interdependent. The plants are indispensable to man for his life. Nature has provided a complete storehouse of remedies to cure all ailments of mankind. The knowledge of drugs has accumulated over thousands of years as a result of mans inquisitive nature, so that today we possess many effective means of ensuring health care.

The human being appeared to be affiliated with more diseases than any other species. There can be little doubt that he very early sought to alleviate sufferings from injury and diseases by taking advantage on the plants growing around him. In the past almost all the medicines used were from plants, being mans only chemist for ages. The history of herbal medicine is as that as old as human civilization.[1]

Diseases are born and drugs came to existence since a very early period to remove the pain of diseases and to cure them. Thus the history of medicines is as old as man kind [2]

In spite of the over helming influence and our dependence on modern medicine and tremendous advances in synthetic drugs, a large segment of the world population (80 % of people) cannot afford the products of the western pharmaceutical industry and has to rely upon the use traditional medicines, which are mainly derived from plant material. The fact is well recognized by the WHO which has recently compiled an inventory of medicinal plants listing over 20,000 species. [3]

Among the 20,000 medicinal plants listed by the WHO over one hundred botanicals are reported to have consistently large demands and are traded in major drug markets of the world. The medicinal virtues of these plants have been worked out and have inclusions in national pharmacopoeias in different countries. Plant cells are now considered to be chemical factories synthesizing a large variety of chemical compounds and thus as a very important renewable source for the production of a variety of chemicals and drugs[4].

There is a reluctance observed in accepting herbal remedies by modern system because of the lack of documentation regarding the so called scientific validity and quality. [5] Traditional use of herbal medicine is the very basis and integral part of various cultures, for thousands of years. Plant based drugs (natural drugs) may be used directly i.e. they may be collected, dried, and used as therapeutic agents (crude drugs), or their chief constituents or active constituents are separated by various chemical processes which are employed as medicines. These active constituents are manufactured chemically to produce the synthetic drugs. The large and growing dependence on synthetic drugs are mainly due to their rapid action. The fast and continuing depletion of natural resources and plant wealth has only added to this dependence on synthetic drugs[6].

Development of traditional herbal medicine into a modern drug of great therapeutic importance is exemplified by the wonder herb *Rouwolfia serpentina, the* root of this has been used for centuries in traditional herbal medicine as a cure for insanity, epilepsy and high blood pressure. There are number of other herbs which have come to light in recent years with miraculous therapeutic effects. The *rosy perivincle*

*(*Vinca roseae) from Madagascar tropical forests provided the million-dollar weapon *Vinblastine* to fight child hood leukemia, a type of blood cancer in infants. *The Pacificyaw* (Taxus buccata) preserved and used by the indigenous ethnic communities of the Pacific island gives the million dollars worth anticancer drug *Taxol*.[7]

India and Herbal Medicine

Geographically our country is situated in the tropical zone. On account of her size, India is the home to a variety of environment from high snow capped mountains to tropical range forests, from cold/hot deserts, scrub lands to lush and fertile plain and valleys, so also mangroves and sea shores. This environment promotes a great variety of habitat for India's rich plant life. It is reported that almost every plant family in the world is represented in India's rich flora-plant life.[8]

India is perhaps the largest producer of medicinal herbs and is rightly called the ***'Botanical garden of the world'***. Medicinal herbs have been in use for thousands of years in one form or other in the Indigenous system of medicine like **Ayurveda, Siddha and Unani.**[9] The medical treatment using plants is date back to ancient times of **Charaka** and **Sushruta** has always been preserved in the popular medicines.[10]

Ayurveda the indigenous system of medicine dating back to the Vedic ages (1500-800B.C.) has been an integral part of Indian culture. The concept of drug in Ayurveda is slightly differing from that in modern medicine.

The term drug is derived from the French word *'drogue'* (a dry herb) is defined as any substance or product used to modify or explore physiological systems or pathological conditions for the benefit of the

recipient. Ayurveda by and large is an experience with nature and unlike the western medicine many of the concept eludes scientific explanations in the modern sense of the term.

In Ayurveda drugs are generally called *'ausadha'* or *'bhesaga*', which means that which will cures pain and sorrowful experience. The source plants of the drugs are called *'ausadha'* even though this term is sometimes used loosely for plants in general. There are three different types of drugs in Ayurveda. They are:

1. **Audbide-** obtained from plants.
2. **Jangama-** obtained from animals.
3. **Parthiva-** obtained from minerals, salts etc…

Of all these plant drugs forms the lion's share of Ayurvedic drugs. With the rapid depletion of our forests impairing the availability of raw drugs, Ayurveda like other systems of herbal medicines, have reached a very crucial phase. About 50% of the tropical forests, the treasure house of plants and animal diversity has already been destroyed and the remaining half may not stand the onslaught of man for another decade. In India alone about 55, 2000 sq.km of the forests we had in 1975 have been reduced to 45, 7000 sq.km by 1982. Forests in India have been disappearing at an average rate of 1.5 ha every year and what is presently lift is only about 8% as against a mandatory 33% of the geographical area. This want on destruction had rendered almost 3-4 thousands of Indian plants on the verge of extinction. The extensive forest destruction has resulted in the extinction of many valuable medicinal herbs along with many others, which would have been potentially useful later.

Higher plants are still the sleeping giants of drug developments, a virtually untapped reservoir of potentially useful source of drugs that will continue to serve mankind in the 21st century as they have done since the dawn of history.

In India alone out of the 15,000 species of higher plants that we have, the number of species that used in medicine is well below one fifth of it.[11]

Among the 19 lakhs of varieties of the vegetable kingdom, 3300 varieties are marked for medicinal purposes that are scientifically grouped under six main species, capable of yielding six varieties of tastes.

The six species are

1. Trees
2. Shrubs
3. Creepers
4. Juicy plants
5. Juiceless plants and
6. Milk yielding plants.

One may wonder what is the remarkable thing in siddha system that is capable of curing many chronic and degenerative diseases by using certain Indian plants. [12]

In the past people had the first had information of the healthy as well as the toxic effect of the various species of the flora and they provided the raw materials to the medical man to compile his Materia Medica. God has created man as well as medicine from the earth. A man

who identifies himself with the laws of nature is free from pains. The speciality of siddha system is its non differentiation of medicines and food. Most of our dietary items such as vegetables, fruits, roots and greens were used in ancient period for a definite reason based on its medicinal use. In Siddha there are about 108 drugs, many among them are used in our daily life and among them are onion, garlic, ginger, lemon, potato, nelli etc…

Saint Thiruvalluvar speaks of the importance of prevention rather than cure. By appropriately including Kalpa drugs in our routine, we are sure to gain the most valuable wealth- the health. The Siddha system of medicine is till today a living science. It has survived through centuries. [13]

Future of plant drugs

The plant kingdom has long supplied us with large number of excellent drugs. But solid scientific research in this field is languishing today and some have explored concern for its future. American health food stores sold more than $190 million worth herbs in 1985 and books and pamphlets describing the putative use of these products amounted to another $33 million in sales. Research and development in the field of herbals continue to flourish in Germany. There new plant drug preparations (Phyto pharmaceuticals) and even new plant constituents are continually being introduced into the market by a relatively large number of manufacturers. A survey there showed that nearly 76% of women interviewed drank herbal teas for their beneficial effects and about 52% of them turned to herbal remedies for their initial treatment of minor illness.

Within the next quarter century the achievement of science and technology will be so great that, when brought to bear upon the mysteries of nature that have long puzzled us, those mysteries will yield their secrets with amazing rapidity. It will be a fascinating and eventful period. We will know not only the cause of disease but also the cure for most. The plant and animal kingdom will continue to save mankind in the 21st century just as they have done since the dawn of the history. Significant new drugs of natural origin and new methods of producing them will continue to be important parts of that service.[14,15]

Anti-inflammatory Herbs[16]

Inflammation is a key feature in autoimmune disease. In some conditions, such as Hashimoto's thyroiditis, inflammation contributes to the disease process. In other conditions, such as Crohn's disease, inflammation may occur as a result of the disease. Inflammation occurs as the immune system reacts to injury, infection, environmental agents, malignancy, and cellular changes. In skin, inflammation is most visible because it causes noticeable swelling, redness, discomfort and pain. The process leading to inflammation, which is known as the inflammatory response, also induces changes that aren't seen but influence the effects of inflammation and their severity.

The inflammatory response is a complex cascade of steps that include an activation of white blood cells, the release of immune system chemicals such as complement and cytokines, and the production and release of inflammatory mediators and prostaglandins. Inflammation may be acute or chronic or relapsing-remitting depending on the disease course. Most conventional treatments for autoimmune disease, including corticosteroids, work by reducing or suppressing inflammation.

Many herbs also possess anti-inflammatory (also known as antiphlogistic) characteristics. Herbs can be used as the sole therapy in autoimmune disease or as complementary corticosteroid-sparing therapies allowing patients to take smaller doses or shorter courses of corticosteroids. Treatment protocols today often rely on both alternative and conventional treatment options in a discipline known as integrative medicine.

Herbal medicine relies on active plant chemicals with biological properties. Many conventional medicines are synthetic compounds designed to mimic the action of plant chemicals. For instance, the heart medication digoxin is derived from the foxglove plant. In herbal medicine, active chemicals are extracted from the plant parts (stems, seeds, roots, or leaves) that are the richest sources. The active chemicals can be quantitatively measured and prepared in the form of capsules, tinctures, teas, tonics, oils, or poultices. Aromatic herbs such as lavender can also benefit the immune system when used topically or as healing oils.

Other herbs known to reduce inflammation include ginger, turmeric, pokeroot, cleavers, devil's claw, liquorice, autumn saffron, boswellin, curcumin, arnica, bromelain, German chamomile, licorice, white willow, witch hazel, and capsaicin.

Many plant chemicals are known to reduce inflammation, including the omega-3 essential oil, which can also be derived from

marine sources. Other plant chemicals, which are also known as phytochemicals, that are known to reduce inflammation include carotenoid and catechin, which belong to the plant chemical family known as bioflavinoids.

Diuretics

These are drugs, which cause a net loss of Na^+ and water in urine. Calomel (mercurous chloride) has been used, as a diuretic from the time of Paracelsus and it was one of the constituents of the famous 'Guy's Hospital Pill'. Organo mercurials given by injection were introduced in the 1920s and dominated for nearly 40 years. The CASE inhibitors were developed in the 1950s from the observation that early sulfonamides caused acidosis and mild diuresis. The first modern orally active diuretic chlorothiazide was discovered in 1957, and by early 1960s its congeners (thiazide diuretics) were already in common use. Availability of frusemide and ethacrynic acid by the mid 1960s revolutionized the pattern of diuretic use. The K^+ sparing diuretics spironolactone and triamterene were developed in parallel to these**.** Among the several plants, *Dolichos biflorus, Tribulus terrestris, Dendrophthoe falcate, Boerhaavia diffusa, Saccharum officinarum, Butea frondosa, Boerhaavia repens, Boerhaavia rependa, Homonia riparia* have shown excellent diuretic activity.[17]

- Diuretics effective for the treatment of oedema have been available since the 16th century.
- Mercurous chloride was known by Paracelsus to be diuretic.

- In 1930, Swartz discovered that the antimicrobial sulfanilamide could be used to treat oedema in-patients with congestive heart failure due to an increase in renal excretion of Na^+.
- Most modern diuretics were developed when side effects of antibacterial drugs were noted, which included changes in urine composition and output.
- Except for spironolactone, diuretics were developed empirically, without knowledge of specific transport pathways in the nephron.

There are numerous worldwide traditional plant remedies. But some of them are most scientifically proven. Physicians and patients are in need of effective therapeutic agents with a low incidence of side effects so, after herbal treatment, the patient come pit heal there in sharp contrast with other acclaims system of medicine.[18, 19]

Fig. no. 1. An aerial view of plant *Cardiospermum helicacabum, Linn.*

1.2 BOTANICAL AND ETHNOMEDICINAL INFORMATION

1.2.1 Introduction

Arrangement of plant into groups and subgroups is commonly spoken as classification. Various systems of classifying plants have gradually developed during past few centuries, which have emerged as a discipline of botanical science known as taxonomy or systemic botany. The word 'taxonomy' is derived from two Greek words 'Taxis' meaning as arrangement and 'Nomas' meaning laws. Therefore, the systemization of our knowledge about plants in an orderly manner becomes subject matter of systematic botany. [1]

The aim and objective of taxonomy is to discover the similarities and differences in the plants, including their closed relationship with their descents from common ancestry. It is a scientific way of naming, describing and arranging the plant in an orderly manner. [2]

Microscopy method allows more detailed examination of a drug it can be used to identifying the organized drugs by their known histological characters. It is mostly used qualitative evaluation of organized crude drugs in entire and powdered form. Ash values, extractive values study and foaming index are used for the study of physical properties.

1.2.2 Synonym [20]

1. *Balloon vine*.
2. *Love in a puff.*

1.2.3 Taxonomical Classification [20]

Kingdom	-	Plantain
Subkingdom	-	Tracheobionta
Super division	-	Spermatophyta
Division	-	Magnoliophyta
Class	-	Magnoliophyta-Dicotyledons
Subclass	-	Rosidae
Order	-	sapindales
Family	-	Sapindaceae- soapberry family
Genus	-	*Cardiospermum L.*
Specie	-	*Cardiospermum halicacabum L.*
Botanical name	-	*Cardiospermum helicacabum L.*

1.2.4 Various Species of the Genus [21]

- *Cardiospermum coridum L.*
- *Cardiospermum grandiflorum Sw.*
- *Cardiospermum halicacabum L.*
- *Cardiospermum halicacabum var. halicacabum*
- *Cardiospermum halicacabum var. microcarpum (Kunth) Blume*

Fig.no. 2 An aerial view of Plant *Cardiospermum helicacabum (Linn.)*

1.2.5 Vernacular Names [22]

Sanskrit	-	Kakadani, Karnasafota, Tejavati, Tejovati,
Hindi	-	Kanphota, Lataphatki.
English	-	Balloon vine
Bengali	-	Lataphatkari, Shibjhul.
Kannada	-	Agniballi, Kanakaia, Kangu.
Malyalam	-	Jayotishmati, Lata, Ulinna, Ulina.
Marathi	-	Kanphuti, Shibajal, Kapalaphodi.
Oriya	-	Sakralata
Tamil	-	Kottavan, Modikkottan, Mudakattan.
Telgu	-	Buddakakara, Ekkudutige, Patalitivva.

1.2.6 Family [22]

Spindaceae- Soapberry family.

1.2.7 Description of Plant [23, 24]

Cardiospermum halicacabum L. is annual or perennial Climber.

3.5m in height, ascending up to 1200m. It is a tendril bearing climbing herbs.

Leaves

Leaves are deltoid, bit ternate, 3-8cm long .leaflets deeply cut, acuminate laterals oblong or ovate, terminal rhombo idianceolate.

Flowers

Flowers are white in umbellate cymes, 3-4 mm long, and peduncles slender stiff, 3-8-10 cm long provided beneath the cyma.

Petioles

Petioles are very acute at the apex and narrow at the base.

Seeds

Seeds are globase black, smooth 4-6mm with a small white heart shaped.

Capsules

Capsules are pyriform and shortly stalked.

Branches

Branches are slender, striate, pubescent or glabrous.

Parts used

Leaves, root and seeds.

Ethno medicinal uses: [25, 26]

1. Diaphoretic.
2. Diuretic.
3. Emetic.
4. Laxative.
5. Refrigerant, anti infertility.
6. Rubefacient and Stomachic.

2. LITERATURE REVIEWS

Systemic literature survey is the first and most important step for the proper selection of plants and it also forms basis for the planning of any scientific work that is to be performed. Due to this reason, the review of literature regarding *Cardiospermum helicacabum Linn.*

- N.T. Modi et al., showed an immediate fall in B.P. in anaesthetized dogs using essential oil obtained from the dried ethyl alcohol(75%) extracts of the seeds of *Cardiospermum helicacabum,Linn.*[27]

- **S.D.** Shukla and et al., reported that the alkaloidal fraction from *Cardiospermum helicacabum, Linn*. . showed in-vitro antibacterial action against some pathogenic organisms. It also causes transient hypotension and cardiac inhibition in anaesthetized dogs.[28]

- A study on photochemical investigation of the leaves of the plant *Cardiospermum helicacabum, Linn.* dealt with isolation and identification of a plant sterol, the Beta-sitosterol and a glycoside, Beta-Sitosterol, D- glucoside from the petroleum ether and ethyl acetate extracts. The presence of alkaloid was observed in the alcoholic extract. The aqueous extract showed the presence of the plant acid, oxalic acid and a group of amino acids. An ash value determination showed the presence of inorganic salts.[29]

- T. Boonmars et al., reported that the extracts of *Cardiospermum helicacabum, Linn.* was effective against third stage larvae of *Strongyloidesster coralis*. The viability of *S. stercoralis* larvae was significantly reduced when exposed to the extracts of *Cardiospermum helicacabum,Linn.*(Antiparasitic activity)[30]

- K.Srinivas. et al., was studied that the entire shrub of *Cardiospermum helicacabum,Linn.* for its phytochemical profilke for two crystalline compounds namely sitosterol beta-D-glucoside and stigma sterol beta-D-glucoside from this shrub well isolated.[31]

- Jigna Parekh, et.al carried out studies on efficacy of aqueous and methanolic extracts of some medicinal plants for Potential Antibacterial activity.[32,33]

- Ragupathy.et.al exploring Ethanobotanical classification for Novel alternative medicine: A case study of *Cardiospermum helicacabum Linn.*(Modakathon, Ballon vine) as traditional herb for treating rheumatoid arthritis.[34]

- Ujjwal Neogri,et.al. reported that the lipid content and in vitro anti microbial activity of oil seeds of some Indian medicinal plants.[35]

- N. Venkat Rao,et.al evaluate the antidirrhoeal activity of whole plant extracts of *Cardiospermum helicacabum,Linn.* in rats.[36]
- Sheeba, M.S.et al., reported that the extract of *Cardiospermum halicacabum.Linn.* is active in ethanol-induced gastric ulcers in rats.[37]

- Waako, P.J.et al.,reported that the extract of *Cardiospermum helicacabum ,Linn.* and *[Momordica foetida] Schumch*. EtThonn. was active as antibacterial activity in vivo and in vitro.[38]

- Gopalakrishnan C. et al., showed that the extraction with different solvents found that the extract of *Cardiospermum helicacabum,Linn.* contains largely tannins, saponins and traces of alkaloids.[39]

- Thomas E. et al., reported that the methanol extracts of twenty one plant species belonging to different family have been screened for in vitro antibacterial activity against multi resistant bacterial isolates including Gram positive and Gram negative strains. *Adhatoda vasica, Cardiospermum helicacabum, Euphorbia hirta,Murraya koenigii, Oldenlandia corymbosa and Phyllanthus niruri* are the plants showing maximum activity.[40]

- Absar A. Qureshi. et al., reported that the ethanolic extracts of the whole plant of *Cardiospermum helicacabum,Linn.* showed anti infertility activity at a dose of 250 mg and 500 mg/kg body weight /

day orally for day 1 to 7 of pregnancy. It showed significant decrease in implantation.[41]

- P.Murugan.et al., reported that the extract with different solvents of five plant species i.e. *Cardiospermum helicacabum L, Cissampelos pareiral, Bauhira purpurea L, Rhinacanthus nastus,(L), Kurzvar nastus and Swertia corymbosa* were screened for in vitro antibacterial activity against different species of bacteria. Most of the activity was against Gram positive bacteria.[42]

- M. Premanathan et al., reported that the extract of *Cardiospermum helicacabum,Linn.*(Shoot+ fruit) and *Cinnamomum cassia* (bark) shows most effective against HIV.[43]

- Raman N. et al., repoted that the extract of the whole plant of *Cardiosprmum helicacabum, Linn.* Shows antibacterial activity against human and Plant pathogens.[44]

3. RESEARCH ENVISAGED

3.1 AIM OF PRESENT STUDY

We planed here study like plant is *Cardiospermum helicacabum Linn.* in detail due to its effectiveness as analgesic, anti-inflammatory, diaphoretic, diuretic and antibacterial.

Traditional use of this plant in India includes consumption as a vegetable, fodder and various medicinal remedies to treat chronic bronchitis, snake bites, or as diuretic, stomachic and rubefacient. The juice of the herb is claimed to cure earaches and to reduce hardened tumors.

Looking to the scope of herbal drug and increasing demand especially in disease of liver, hypertension, diabetes, diuretic, cancer, renal diseases, inflammation, infectious diseases, arthritis and skin disease etc., hence, it is planned here to study the plant like *Cardiospermum helicacabum,Linn.* The selection of the plant *Cardiospermumhelicacabum, Linn*. was made on the basis of its

- Easy availability and wide geographical distribution of the plant globally in almost every climatic condition.
- Therapeutic value
- There is no proper pharmacognostical, phytochemical and pharmacological information regarding the leaves of *Cardiospermum helicacabum, Linn.*
- Degree of research work which is not done.

Keeping in mind about the adverse effects of allopathic drugs like Anti-inflammatory and diuretic, above said activity were studied with different parameters of leaves of *Cardiospermum helicacabum, Linn.* in order to give all possible scientific validation.

The expected outcomes of the present research work are

1. Establishment of Pharmacognostical standards.
2. Establishment of Anti-inflammatory
1. Activity.
3. Establishment of Diuretic
2. Activity.
4. Isolation and characterization of active principles.
5. Qualitative estimation of the active extract

3.2 PLANT COLLECTION AND IDENTIFICATION

3.2.1. Collection of Specimen

The species for the proposed study of leaves of ***Cardiospermum halicacabum, Linn.*** was collected in month of June-July 2008 in Western Sahyadri Ghat region of Maharashtra.

3.2.2. Taxonomical Identification

The species for the study was identified and authenticated as *Cardiospermum helicacabum Linn.* by P.G. Divakar, Joint Director, Botanical Survey of India, Western Circle, Pune-1.
(Certificate No.BSI/WC/Tech/2009/719)

3.2.3. Treatment of leaves

First of all the leaves were washed with water and dried it in sunlight for one hour and then it was dried in shade. By the help of wood grinder the dried leaves was powdered and was passed through the sieve no.60 for powder analysis and coarse powder was used for phytochemical work.

4. PLAN OF WORK

The plan of work of the leaves of *Cardiospermum helicacabum Linn.* was carried out as following

1. **Pharmacognostical studies**
    - A. Macroscopical investigation
    - B. Microscopical investigation
    - C. Physico-chemical constants
        - a. Ash values
        - b.Extractive values
        - c.Loss on drying
    - D. Foaming index
    - E. Fluorescence analysis

2. **Phytochemical studies**
    - A. Purification of solvents
    - B. Preparation of extracts
    - C. Qualitative phytochemical analysis
    - D. Thin layer chromatography
    - E. Spectral studies
        - a.UV spectroscopy
        - b. FT-IR spectroscopy

3. **Pharmacological studies**
    - A**.** Acute oral toxicity
    - B. Anti-inflammatory activity.
    - C. Diuretic activity

PLAN OF WORK

CARDIOSPERMUM HELICACABUM, LINN.

Leaves

Pharmacognostical Studies	**Phytochemical Studies**	**Pharmacological Studies**
A. Macroscopical studies	**A. Purification of Solvents**	**A. Acute oral toxicity studies.**
B. Microscopical studies	**B. Preparation of Extracts**	**B. Anti inflammatory activity.**
C. Physicochemical studies	**C. Qualitative Phytochemical Analysis**	**C. Diuretic activity**
a. Ash values	**D. Thin layer chromatography**	
b. Extractive values	**E. Spectral studies**	
c. Loss on drying	**a. UV spectroscopy**	
D. Foaming index	**b. FT-IR spectroscopy**	
E. Fluorescence analysis		

5. EXPERIMENTAL SECTION

5.1 PHARMACOGNOSTICAL STUDIES

5.1.1 Introduction

Pharmacognostical studies mainly include study of morphological characters, microscopical characters and powder microscopy. It also includes study of physico-chemical constants like ash values, extractive values, and loss on drying of the leaves powder. Microscopical studies of leaves have also been carried out.

The aim and objective of taxonomy is to discover the similarities and differences in the plants, indicating their closer relationship with their descents from common ancestry. It is a scientific way of naming, describing and ranging the plants in ordered manner. A large number of plant families have certain distinguishing characteristics that permits crude from these families to studied at one time.

Microscopy is an important tool in the evaluation of crude drugs which is applicable at various levels such as the authentification of the crude drugs, study of powdered drugs visualizing calcium oxalate crystals.

Microscopy method allows more detailed examination of a drug. It can be used to identifying the organized drugs by their known histological characters. It is mostly used for qualitative evaluation of organized crude drugs in entire and powdered form. Ash values,

extractive values and foaming index are used for the study of physical properties.

5.1.2 Materials and methods

Collection of specimen

Most care was taken to select healthy plant and for normal organs. The required sample of ***Cardiospermum halicacabum, Linn.*** leaves were cut and removed from the plant and fixed in FAA (Farmalin-5ml + Acetic acid -5ml + 70% Ethyl alcohol-90ml). After 24 hrs of fixing, the specimens were dehydrated with graded series of tertiary-Butyl alcohol. Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point 58-60 ºC) until TBA solution attained super saturation. The specimens were cast into paraffin blocks.[46]

5.3.2. Sectioning

The paraffin embedded specimen was sectioned with the help of Rotary Microtome. The thickness of the section was 10-12µm. De-waxing of the sections and the section was stained with Toluidine blue. Since Toluidine blue is a polychromatic stain, the staining results were remarkably good and some cytochemical reactions were also obtained. The dye rendered pink color to the cellulose walls, blue to lignified cells dark green to suberin, violet to the mucilage, blue to the protein bodies etc. Wherever necessary sections were also stained with saffranin and fast green and I-KI (for starch.)[45,46]

For studying the stomatal morphology venation pattern and trichome distribution, paradermal (section taken parallel to the surface of leaf) as well as clearing of leaf with 5% sodium hydroxide or epidermal peeling by partial maceration employing Jeffery's maceration fluid were

prepared and glycerin mounted temporary preparation were made for macerated / cleared materials.

Photomicrographs

Microscopic descriptions of tissues are supplemented with micrographs wherever necessary. Photographs at different magnifications were taken with Nikon Labphot 2 Microscopic Unit. For normal observation bright field was chosen. For the study of crystals, starch grains and lignified cells, polarized light was employed. Since these structures have birefringent property under polarized light they appear bright against dark background. Magnifications of the figures are indicated by the scale bars.[48]

5.1.3 Macroscopic studies

The plant occurs both in wild and cultivation conditions. The leaves, seeds and roots are used as medicine.

The plant is a fast growing weedy, tendril-climber with three cornered bladder type fruits formed from scented white flowers that bloom in summer. Its fruits are heart shaped.

5.1.4 Microscopic studies

Microscopical Features of *Cardiospermum halicacabum L.* leaves are as follows

Lamina (fig. no. 4)

The lamina is 200 – 300µm thick. The abaxial and adaxial surfaces are smooth and even. The lamina is dorsiventral and mesomorphic. The adaxial epidermis cells of varying size and shape. The abaxial epidermis has narrowly cylindrical cells. The mesophyll is differentiated in to abaxial palisade zone and abaxial spongy parenchyma zone.

The palisade zone is 100-150 µm thick. It is 2 or 4 layered, compact. The spongy parenchyma consists of wide lobed 5 or 6 layers of cells which are interconnected from reticulations. The lateral veins are prominent they are collateral and have single layered, hyaline bundle sheath cells. (fig.no.4)

The marginal part of lamina is bleeont; the epidermal layers are similar to the mid part of the lamina. The palisade zone and spongy parenchyma zone continue with margin. The lateral veins are also prominent in the margin (fig.no.4-2). The margin is about 150µm thick.

Midrib (fig.no. 3.2)

In cross sectional view the midrib is more or less biconvex with adaxial hump and abaxial broad conical body. It is 500µm in vertical axis. The adaxial hump is 200µm wide the abaxial part is 350µm wide.

The adaxial hump has collenchymatous group tissue and prominent epidermis with squarish cells. The abaxial midrib also has district epidermal layers of cuticle cells and large parenchymatous growed parenchyma. The vascular stored in singe and bowl shaped. It consists of a few paral files of angular, thick walled xylem elements and an abaxial zone of small clusters of phloem. The vascular strands 200µm horizontacally and 100µm/vertically.

Crystal distribution (fig.no. 4-2, 5-3)

The calcium oxalate crystals are abundant with mesophyll tissue. They are large deruses. They are 20-40 µm in diameter.

Venation pattern (fig.no. 5-1, 2)

The lateral veins are thin and prominent they do not form distinct vein islets. District vein- islets are seen at contains places only. The islets are small or wide they are mostly squarish in shape. The vein determinations district. They are short, thick and mostly unbranched or occasionally branched once.

Stomata (fig.no. 6)

The stomata occur on both surfaces on the leaf. Stomata are more outer lower side than on the upper side. The stomata are predominantly diacytic with two subsidiary cells with their common walls at right angle with long axis of the guard cells the two subsidiary cells may be equal or one of them may be much larger than the other. Some of the guard cells have elliptic aperture with other have slit like closed aperture. The epidermal cells are lobed. The anticlinal walls are thick and much wavy.

Petiole (fig.no. 7-1, 2)

The basal (proximal) part of the petiole is planoconvex with short blant rings. Epidermal layer is thin, but district comprising of small squares cells (fig.no. 7-1) the outer zone of the ground tissue, especially on the adaxial and abaxial parts, the cells are collenchymatous. Remaining inner portion have this walled parenchyma cells. The (distal) upper part of the petiole has long thick rings and spindle strands are two in the proximal part and three bundles in the distal part. The bundle are lop shaped and collatrons. (Fig.no. 7-1, 2)

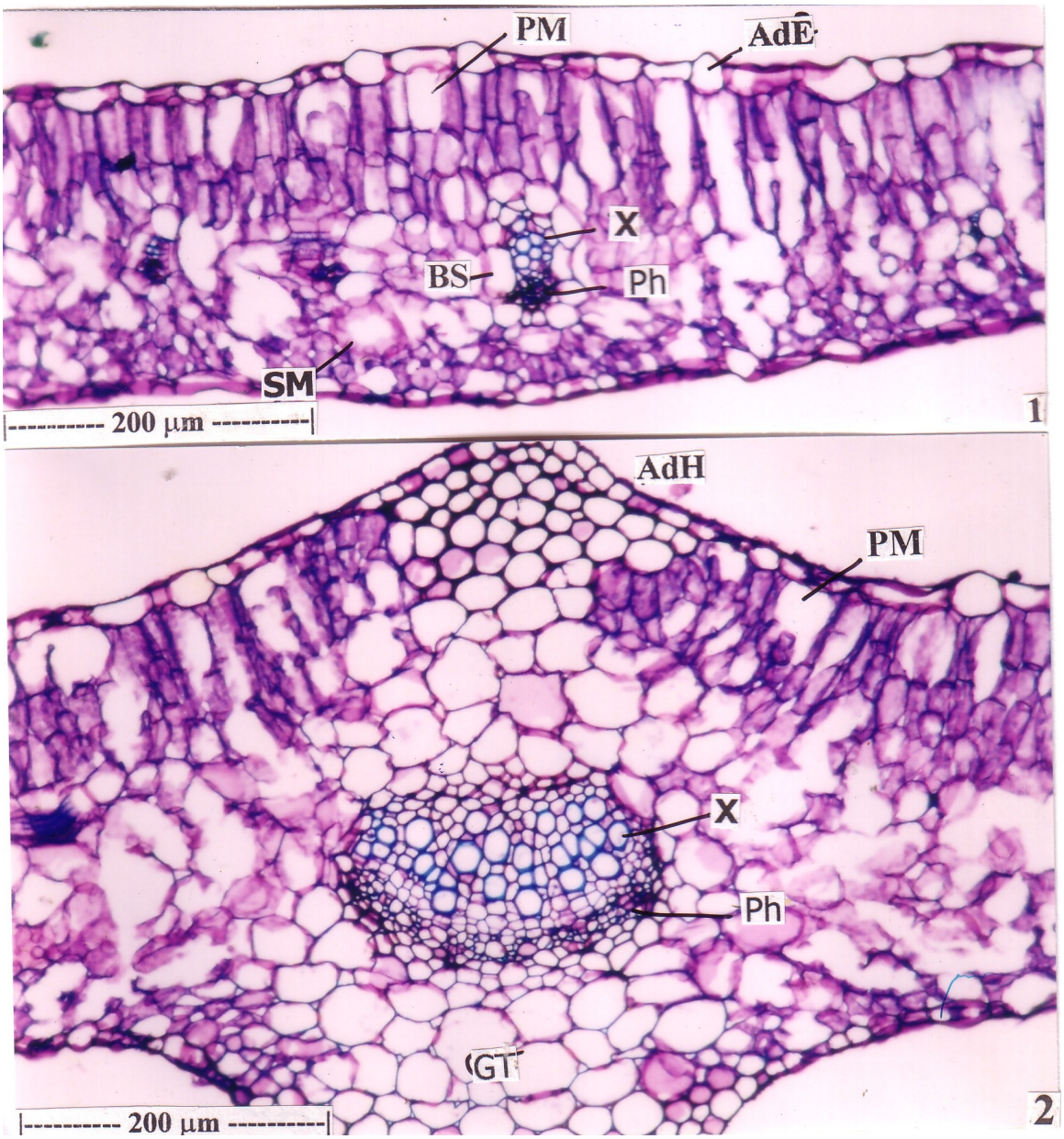


Fig.no. 3.1 T.S of leaf through lateral vein with lamina

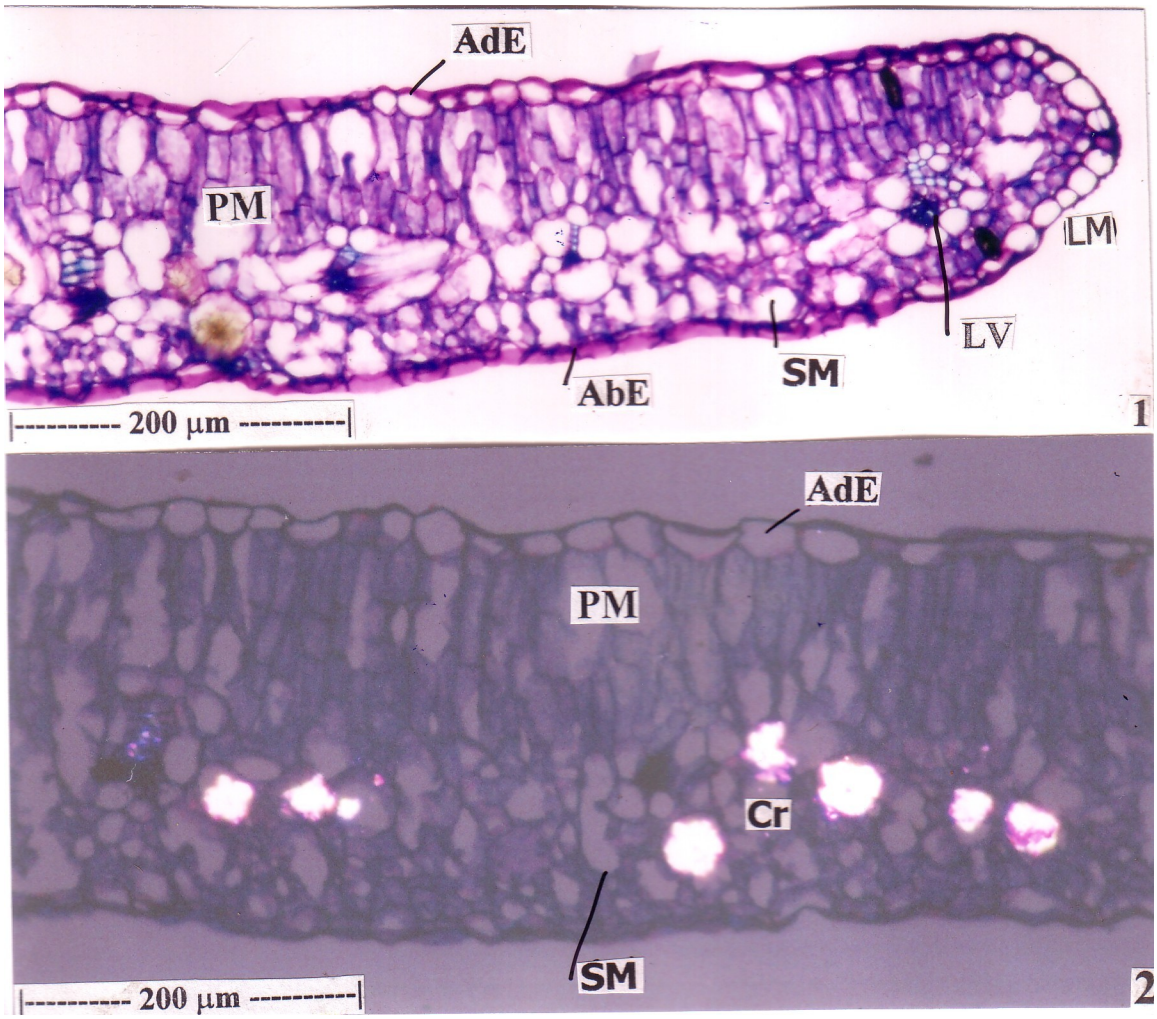
Fig.no. 3.2 T.S. of Mid rib enlarged

[AdE – Adaxial epidermis, **AdH**- Adaxial hump, **BS**- Bundle sheath

GT- Ground tissue, **Ph**- Phloem, **PM**- Palisade mesophyll

SM- Spongy mesophyll, **X**- Xylem]

Fig.no. 4 Anatomy of the lamina

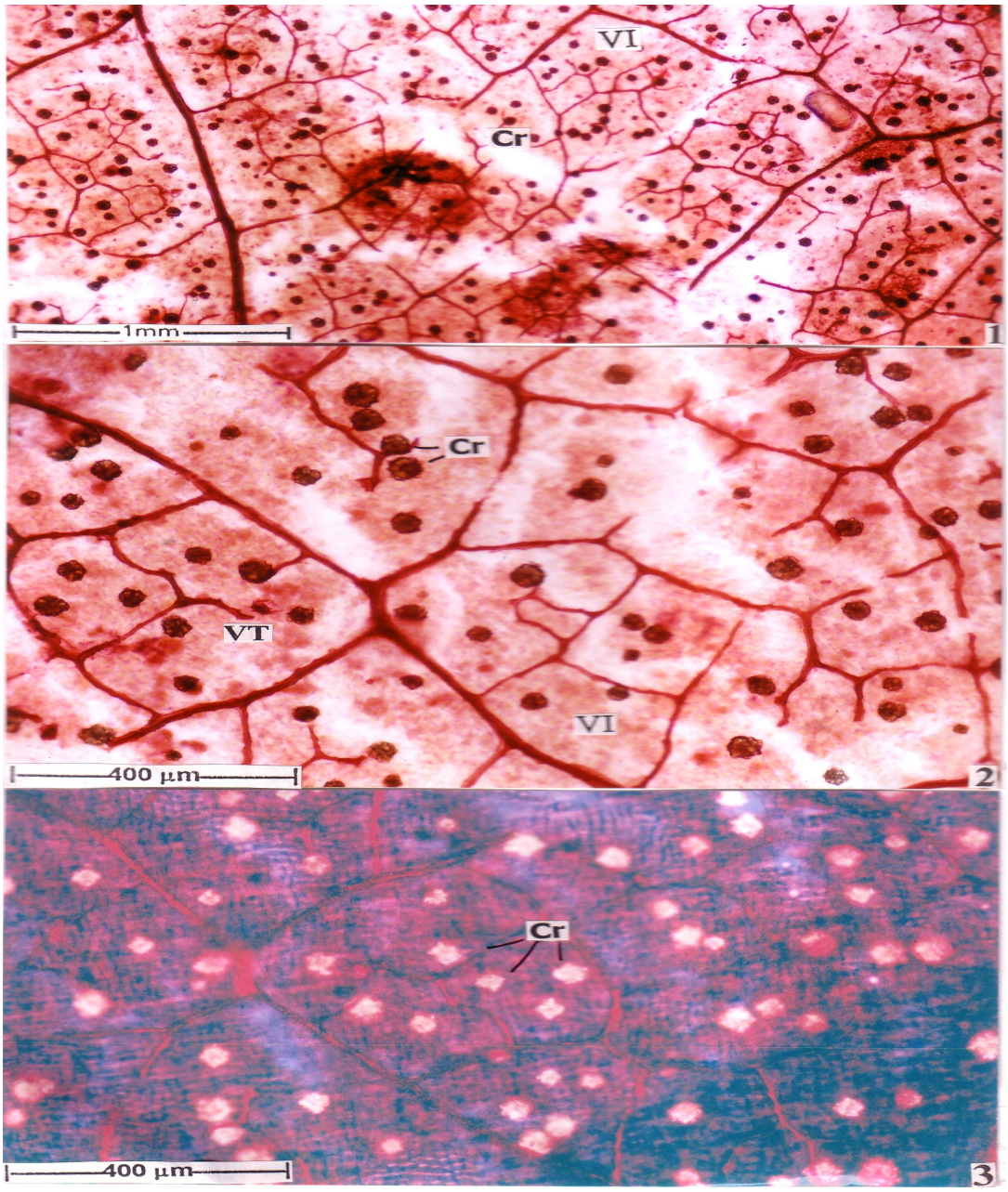


1. T.S of leaf margin.

2. T.S. of lamina showing crystal distribution under polarized light microscopic.

[**AbE** – Abaxial epidermis, **AdE**- Adaxial epidermis, **Cr**- crystals, **LM**- Leaf Margin, **LV**- Lateral vein, **PM**- Palisade mesophyll **SM**- Spongy mesophyll.]

Fig.no. 5 Vein-islets and Vein Termination

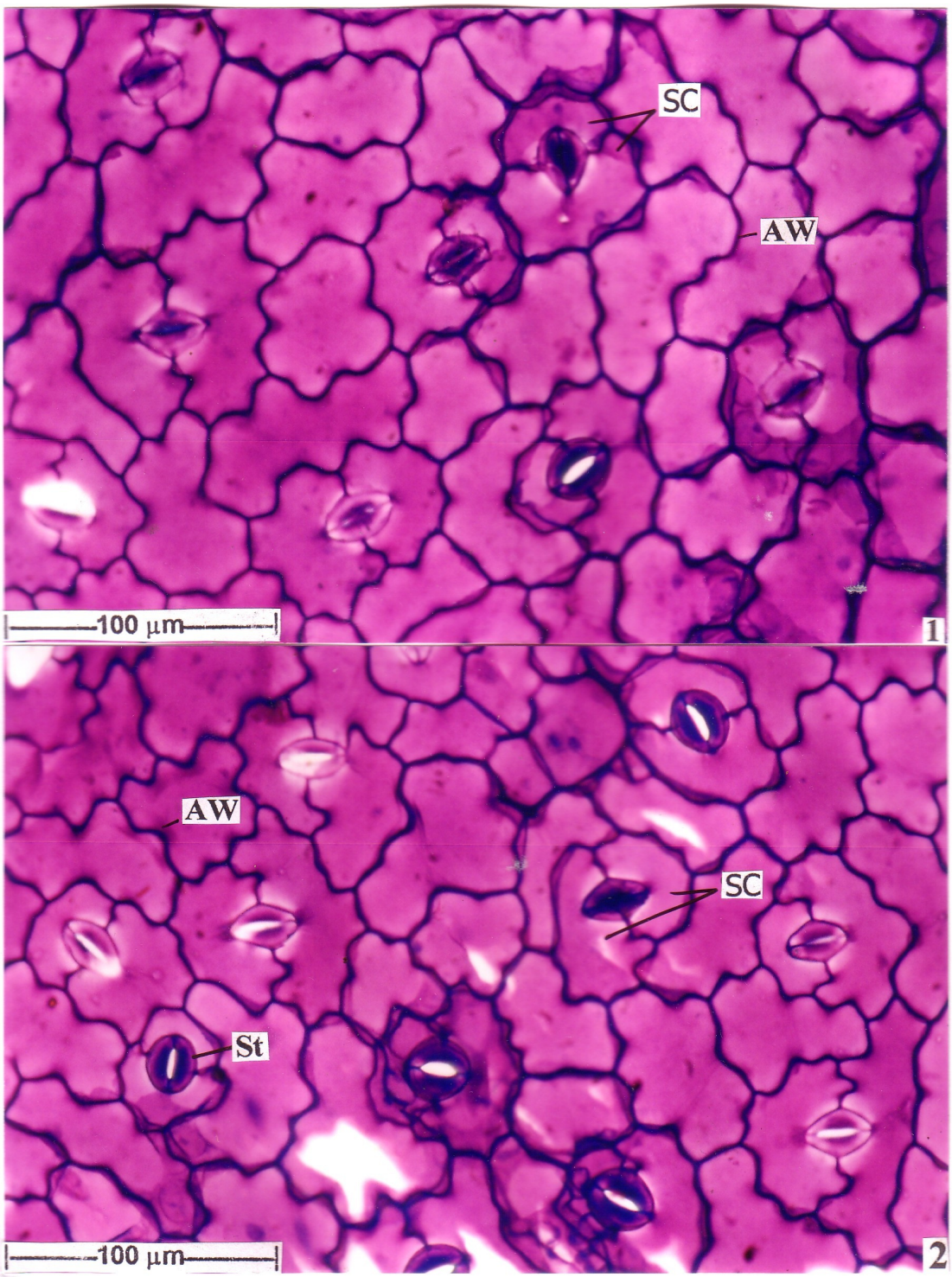


1.Cleared leaf showing vein-islet and vein termination under low magnification.

2. Same as above enlarged.

3. Druses in the lamina as seen under polarized light microscope.

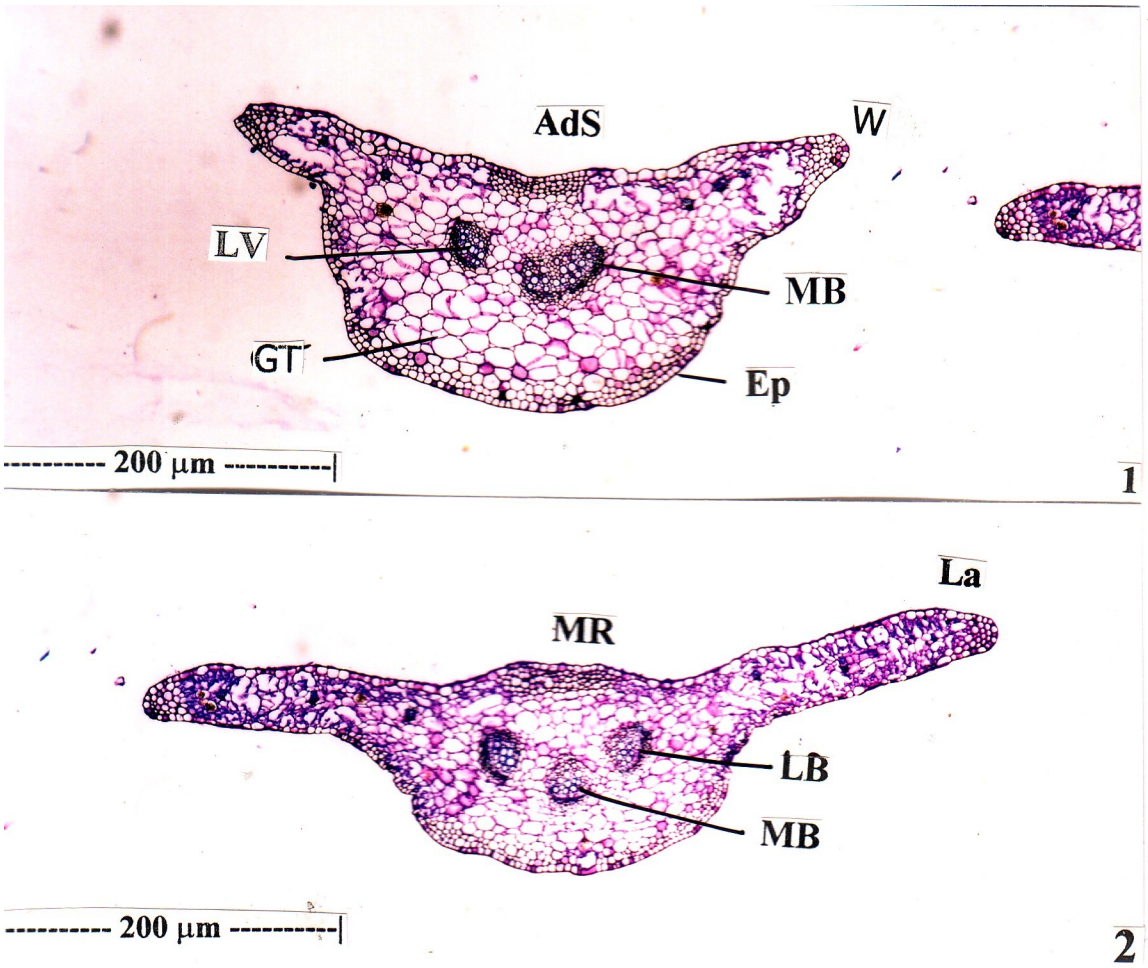
Fig.no.6 Stomatal Morphology



1, 2:- Abaxial epidermis with stomata

[**AW**- Aniclinal wall, **SC**- Subsidiary cells, **St**- Stomata]

Fig.No.7 Anatomy of the petioles



1. T.S of petiole—proximal region entire view.

2. T.S. of petiole—proximal.

[**AdS** – Adaxial side, **Ep**- Epidermis, **GT**- Ground Tissue,

La- Lamina, **LB**- Lateral bundle, **LV**- Lateral Vein,

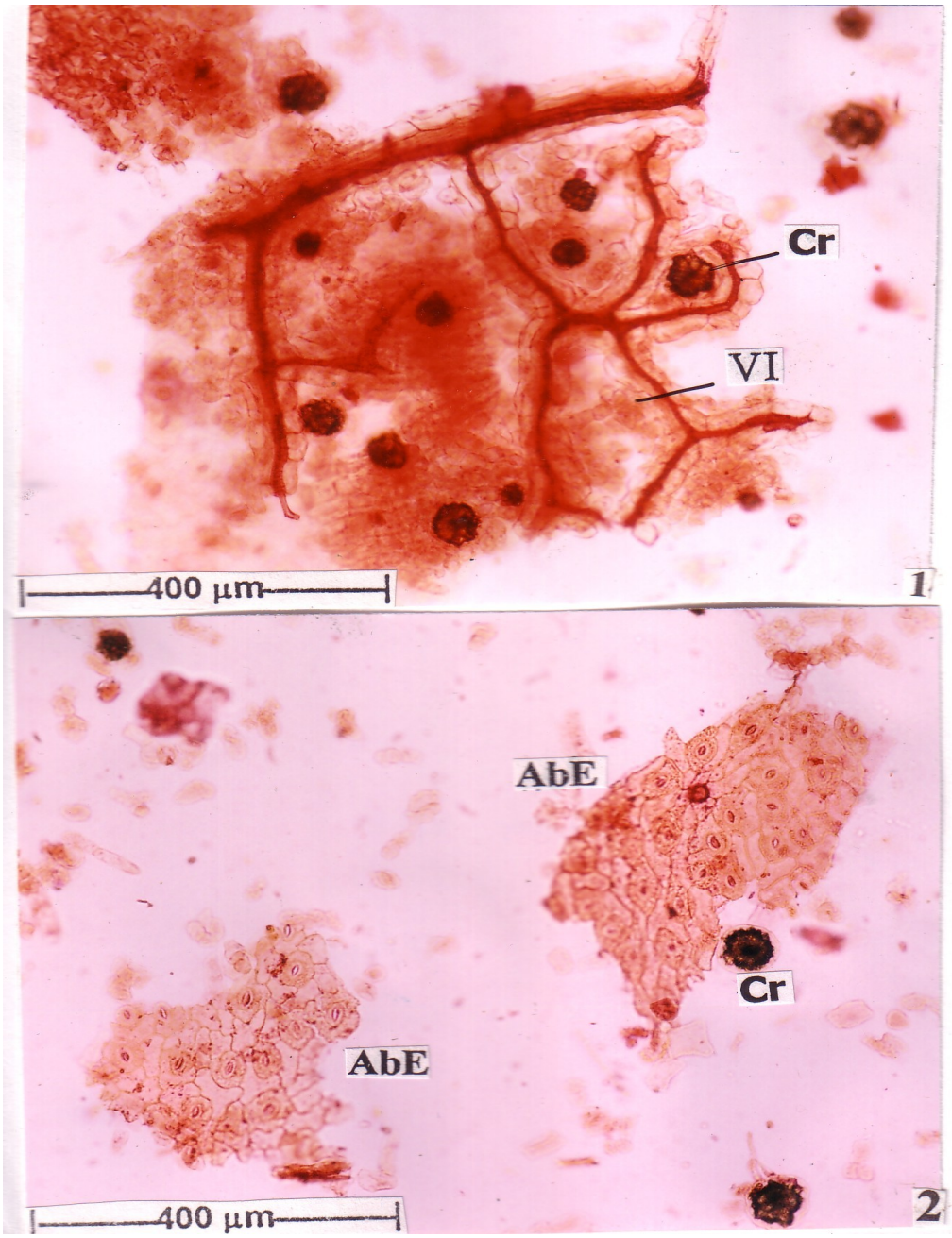
MB- Medial bundle, **MR**- Midrib, **W**- Winy.]

Powder microscopic observations (fig.no. 8)

The powdered leaf exhibits the following element under the microscope:-

- The broken leaf fragment with veinislers and vein terminations are seem the vein terminations are long, wavy and thick the veins are associated with the bundle sheeth parenchyma (fig.no.8-1)
- The fairly large druses are abundant in the powder. They occur within this walled circular parenchyma cells (fig.no.8-1, 2)
- The small pieces of epidermal peeling are seen with powder. Stomata of parasitic type are seem in epidermal fragments (fig.no.8-2)
- The wavy anticlinal walls and amobokd shape of the epidermal cells are other characteristic of the powdered materials.

Fig.no.8 Powder Microscopy of the leaf



1) Crystal and Vein-islets

2) Fragments of Abaxial epidermis

[**AbE** - epidermis, **Cr**- crystals, **VI** - Vein islets.]

5.1.5 PHYSICO-CHEMICAL STUDIES

Ash values

The residue remaining/left after incineration of the crude drug is designated as ash. The residue obtained usually represents the inorganic salts naturally occurring in the drug and adhering to it. It varies with in definite limits according to the soils. It may also include inorganic matter added for the purpose of adulteration. Hence an ash value determination furnishes the basis for judging the identity and cleanliness of any drug and gives information relative to its adulteration/contamination with inorganic matter, thus ash values are helpful in determining the quality and purity of drug.[49]

The total ash of a crude drug reflects the care taken in its preparation. The acid insoluble ash is a part of the total ash that is insoluble in dilute hydrochloric acid. A higher limit of acid-insoluble ash is imposed, especially in cases where silica may be present or when the calcium oxalate content of the drug is very high. Some analyst favors mixing of sulphuric acid with the powdered crude drug before ashing and this sulphated ash value is normally less fusible than ordinary ash.

Procedure given in Indian Pharmacopoeia was used to determine the different ash values such as total ash, acid insoluble ash, water-soluble ash value and sulphated ash.[50, 51,52]

Determination of Total Ash Value

Accurately weighed about 3 gms of air dried powdered drug was taken in a tared silica crucible and incinerated by gradually increasing the temperature to make it dull red hot until free from carbon. Cooled and weighed, repeated for constant value. Then the percentage of total ash was calculated with reference to the air-dried drug (Table no.1)

Determination of Acid Insoluble Ash Value

The ash obtained as directed under total ash was boiled with 25ml of 2 N HCL for 5 minutes. The insoluble matter was collected on an ash less filter paper, washed with hot water, dried the filter paper, ignited and weighed. Then calculated the percentage of acid insoluble ash with reference to the air-dried drug (Table no.1)

Determination of Water Soluble Ash Value

The total ash obtained was boiled with 25ml of water for 5 minutes. The insoluble matter was collected on an ash less filter paper, washed with hot water and ignited for 15 minutes at a temperature not exceeding 450^{o}C. The weight of insoluble matter was subtracted from the weight of total ash. The difference in weight represents the water-soluble ash. The percentage of water-soluble ash was calculated with reference to the air-dried drug. (Table no.1)

Determination of Sulphated Ash Value

About 3 gm. of accurately weighed air dried powdered drug was taken in a tared silica crucible, which was previously ignited and weighed. Then ignite gently at first until the drug was thoroughly charred. The crucible was cooled and residue was moistened with 1ml of

concentrated Sulphuric acid, heated gently until the white fumes were no longer evolved and ignited at 800^{O}C± 25^{O}C until all the black particles had disappeared. The crucible was allowed to cool, few drops of Sulphuric acid was added and again heated. The ignition was carried out as before, allowed cooling and weighed to get a constant weight (difference not more than 0.5 gm between two consecutive readings.) The percentage of sulphated ash was calculated with reference to the air-dried drug. All the ash values were calculated and recorded. (Table no.1)

5.4.2 EXTRACTIVE VALUES

Extractive values of crude drugs are useful for their evaluation, especially when the constituents of a drug cannot be readily estimated by any other means. Further, these values indicate the nature of the constituents present in a crude drug.[50 ,51, 52]

Determination of Alcohol Soluble Extractive Value

5gms of the air dried coarse powder of the leaf of ***Cardiospermum halicacabum, Linn.*** was macerated with 100 ml of 90% ethanol in a closed flask for 24 hours, shaking frequently during the first 6 hours and allowing to stand for 18 hours. Thereafter, it was filtered rapidly taking precautions against loss of the solvent. Out of that filtrate, 25ml. of the filtrate was evaporated to dryness in a tared flat-bottomed shallow dish, dried at 105^{o}C and weighed. The percentage of ethanol soluble extractive value was calculated with reference to the air-dried and the results were recorded. (Table no.1)

Determination of Water soluble Extractive Value

Weigh accurately the 5gms of coarsely powdered drug and macerate it with 100ml of chloroform water in a closed flask for 24 hours, shaking frequently during the first 6 hours and a having to stand for 18 hours. Thereafter, it was filtered rapidly taking precautions against loss of the solvent. Then 25 ml of the filtrate was evaporated to dryness in a tared flat bottomed shallow dish, dried at 105°C and weighed. The percentage of water-soluble extractive was calculated with reference to the air dried drug and the results were recorded. (Table no.1)

5.4.3 LOSS ON DRYING

Loss on drying is the loss in weight in% w/w determined by means of the procedure given below. It determines the amount of volatile matter of any kind (including water) that can be driven off under the condition specified (dessicator or hot air oven). If the sample is in the form of large crystals, then reduce the size by quickly crushing to a powder.

Procedure

About 2 gm. of powdered drug was weighed accurately in a tared porcelain dish, which was previously dried at 105°C in hot air oven to constant weight and then weighed. From the difference in weight, the percentage loss of drying with reference to the air dried substance was calculated and recorded. [50, 53] (Table no.1)

Weight of dried leaves powder	: 2gm
Weight of dried leaves powder at 105^0 C	: 1.78 gm.

Percentage loss on drying : 10.93% w/w.

TABLE NO.1

PHYSICO-CHEMICAL PARAMETERS OF POWDERED

5.1.6 FOAMING INDEX

Foaming index is mainly performed to determine the saponin content in an aqueous decoction of plant material.

Determination of foaming index

Weighed accurately about 1gm of coarsely powdered drug and transferred to 500ml conical flask containing 100ml of boiling water. Maintained at moderate boiling at 80-90^{o}C for about 30 minutes. Cooled and filtered into a volumetric flask and added sufficient water through the filter to make up the volume to 100ml (V_1). Cleaned 10 stopper test tube of uniform dimensions were taken and marked from 1 to 10. Measured and transferred the successive portions of 1, 2, 3 ml up to 10ml and adjusted the volume of the liquid in each tube with water to 10ml. Stoppard the tubes and shaken them in a lengthwise motion for 15 seconds uniformly and allowed to stand for 15minutes and measure the

height. If the height of the foam in every tube is less than 1cm, the foaming index is less than 100 (not significant). The foam was more than 1 cm height after the dilution of plant material. The corresponding number of the test tube was the index sought, if the height of the foam in every tube is more than 1 cm; the foaming index is mote than 1000. In this case, 10ml of the first decoction of the plant material is measured and transferred to a 100ml volumetric flask (V_2) and volume is made to 100ml and followed the same procedure.[50, 54, 55]

$$\text{Foaming index} = \frac{1000}{a} \quad (V_1)$$

$$\text{Foaming index} = \frac{1000}{a} \times 10 \quad (V_2)$$

Where, a = volume (ml) of decoction used for preparing the dilution in the tube where exactly 1 cm or more foam was observed.

The foaming index was calculated by using this formula and was tabulated. (Table no.2)

$$\textbf{Foaming index} = \frac{1000}{a}$$

$$= \frac{1000}{5}$$

$$= 200$$

Thus, the foaming index of the powdered leaves of ***Cardiospermum halicacabum* ,*Linn*.** was found to be 200.

TABLE NO. 2

FOAMING INDEX OF THE POWDERED LEAVES OF *CARDIOSPERMUM HELICACABUM, LINN.*

5.1.7 FLUORESCENCE ANALYSIS [49, 53, 54]

In the near ultraviolet region of the spectrum (150–300 nm) some of the phytoconstituents show more or less brilliant coloration when

exposed to radiation. This phenomenon of emitting visible wavelengths as a result of being excited by radiation of a different wavelength is known as fluorescence. Sometimes the amount of ultra violet light normally present with visible light is sufficient to produce the fluorescence, but more often a more powerful source of ultra-violet light is necessary, e.g a mercury vapour lamp. It is often possible to make use of this phenomenon for the qualitative examination of herbal drugs.[52,57]

Fluorescence analysis of the powdered leaves of *Cardiospermum helicacabum, Linn.* was observed in daylight and UV light. Also the fluorescent study was performed on treating the drug powder with different chemical reagents. The observed results were given in the table no.3

TABLE NO. 3

DATA FOR FLUORESCENCE ANALYSIS OF POWDERED LEAVES OF *CARDIOSPERMUM HELICACABUM, LINN.*

Sr. No.	Chemical Treatment	Day light	UV Light
			254 nm
1.	Powder as such	Pale Yellow	Dark brown
2.	Powder + Water	Pale brown	Dark brown

3.	Powder + 1 N HCl	Dark Reddish Brown	Dark green
4.	Powder + 5%NaOH	Light Reddish Brown	Dark Greenish brown
5.	Powder + 1 N NaOH	Orange	Dark Greenish brown
6.	Powder + 50% HNO_3	Yellow	Dark green
7.	Powder + 50% H_2SO_4	Dark Brown	Dark Reddish
8.	Powder +Picric acid	Dark Yellow	Dark Greenish
9.	Powder +Acetic acid	Dark Brown	Dark Brown
10.	Powder + NH_3+HNO_3	Yellow	Dark Brown
11.	Powder + $FeCl_3$	Light Brown	Dark Greenish brown

5.2 PHYTOCHEMICAL STUDIES

5.2.1 Introduction

Plants may be considered as biosynthetic laboratories in which various kinds of organic compounds are synthesized such as Carbohydrates, Proteins, Lipids, Flavonoids, Glycosides, Alkaloids, Volatile oils, and Tannins etc., which exert a physiologic effect and are utilized as biologically active components by men since time immemorial. The medicinal value of any plant drug however depends on

the nature of chemical constituents present in it and is referred to as active principle.

For our present study, we had taken of ***Cardiospermum halicacabum, Linn.*** powdered leaves to extract the active constituents are tested the phytochemical constituents present in them.

For the isolation of compounds, we were using chromatographic techniques by developing various solvent systems and confirm the active compounds present in them by different spectral studies including UV and FT-IR spectroscopy

5.2.2 Purification of solvents [55, 56]

Ethyl alcohol

A dried round bottom flask was fitted with a double surface condenser and a calcium chloride guard tube. Dry magnesium turnings (5gm) and iodine (0.5gm) were placed in the flask followed by 50-75ml of commercial absolute alcohol. The mixture was warmed until the magnesium is converted to ethanolate, then 900ml of commercial absolute alcohol was added and refluxed for 30 minutes. The ethanol is directly distilled into vessel and used.

Distilled Water

Water obtained by distillation is used for aqueous extraction of powdered drug material.

5.2.3 Preparation of extracts

Preparation of the extracts of powdered root and leaves of ***Cardiospermum halicacabum, Linn.*** by using following solvents:

(a) Ethyl alcohol

(b) Distilled Water

(a) **Alcoholic extract**

The shade dried coarse powder of the leaves (500gm) was packed well in soxhlet apparatus and was subjected for continuous hot extraction with 99.9% ethanol until the completion of the extraction. The extract was filtered while hot and the resultant extract was distilled in vacuum under reduced pressure in order to remove the solvent completely. Dried and kept in a desiccator till experimentation. Obtained extract (dark brownish) was weighed and percentage yield was calculated in terms of air-dried powdered crude material.

(b) Aqueous extract

The shade dried coarse powder of the bark (500 gm) was packed well in soxhlet apparatus and was subjected to continuous hot extraction with distilled water until the completion of extraction. The extract was filtered while hot and the resultant extract was distilled in vacuum under reduced pressure in order to remove the distilled water completely. It was finally dried and kept in a desiccator till experimentation. Obtained extract (dark reddish brown) was weighed and percentage yield was calculated in terms of air-dried powdered crude material.

The yield and % yield of both alcoholic and aqueous extracts of powdered leaves of ***Cardiospermum halicacabum Linn.*** were reported.

TABLE NO: 4

EXTRACTION VALUES OF ALCOHOLIC AND AQUEOUS EXTRACTS OF POWDERED LEAVES OF *CARDIOSPERMUM HELICACABUM, LINN.*

Sr. No.	Extracts	Yield (gms.)	% Yield (w/w)
1.	Alcoholic Extract	42.70	8.54

2.	Aqueous Extract	54.68	10.96

5.2.4 QUALITATIVE PHYTOCHEMICAL ANALYSIS

Both alcoholic and aqueous extracts obtained by the powdered leaves of ***Cardiospermum halicacabum, Linn.*** were subjected to various qualitative tests for the identification of phytoconstituents present.[49,51,55,56]

1. Test for Alkaloids

(a) **Dragendorff's test**: To the 1 ml of extract, add 1 ml of Dragendorff's reagent (potassium bismuth iodide solution). An orange-red precipitate indicates the presence of alkaloids.

(b) **Mayer's test:** To the 1 ml of extract, add 1 ml of Mayer's reagent (Potassium mercuric iodide solution). Whitish yellow or cream coloured precipitate indicates the presence of alkaloids.

(c) **Hager's test:** To 1 ml of extract add 3ml of Hager's reagent (saturated aqueous solution of picric acid) yellow colored precipitate indicates the presence of alkaloids.

(d) **Wagner's test:** To the 1 ml of extract add 2 ml of Wagner's reagent (iodine in potassium iodide) formation of reddish brown precipitate indicates the presence of alkaloids.

2. Test for Saponins

(a) Take small quantity of alcoholic and aqueous extract separately and add 20 ml of distilled water and shake in a graduated cylinder for 15 minutes lengthwise. A 1cm layer of foam indicates the presence of saponins.

3. Test for Glycosides

(a) **Legal's test:** Dissolve the extract in pyridine and add sodium nitroprusside solution to make it alkaline. The formation of pink to red colour shows the presence of glycosides.

(b) **Baljet's test:** To 1ml of the test extract, add 1ml of sodium picrate solution and the yellow to orange color reveals the presence of glycosides.

(c) **Keller-Killiani test:** 1gm of powdered drug is extracted with 10ml of 70% alcohol for 2 minutes, filtered, add to the filtrate, 10ml of water and 0.5ml of strong solution of lead acetate and filtered and the filtrate is shaken with 5ml of chloroform. The chloroform layer was separated in a porcelain dish and removes

the solvent by gentle evaporation. Dissolve the cooled residue in 3ml of glacial acetic acid containing 2 drops of 5% ferric chloride solution. Carefully transfer this solution to the surface of 2ml of concentrated sulphuric acid. A reddish brown layer forms at the junction of the two liquids and the upper layer slowly becomes bluish green, darkening with standing.

(d) **Borntrager's test:** Add a few ml of dilute Sulphuric acid to 1ml of the extract solution. Boil, filter and extract the filtrate with chloroform. The chloroform layer is treated with 1ml of ammonia. The formation of red color of the ammonical layer shows the presence of anthraquinone glycosides.

4. Test for Carbohydrates

(a) **Molisch's test:** To 2ml of the extract, add 1ml of α-napthol solution, add concentrated sulphuric acid through the side of the test tube. Purple or reddish violet color at the junction of the two liquids reveals the presence of carbohydrates.

(b) **Fehling's test:** To 1ml of the extract, add equal quantities of Fehling solution A and B, upon heating formation of a brick red precipitate indicates the presence of sugars.

(c) **Benedict's test:** To 5ml of Benedict's reagent, add 1ml of extract solution and boil for 2 minutes and cool. Formation of red precipitate shows the presence of sugars.

5. Test for Tannins and Phenolic Compounds

(a) Take the little quantity of test solution and mixed with basic lead acetate solution. Formation of white precipitates indicates the presence of tannins.

(b) To 1ml of the extract, add ferric chloride solution, formation of a dark blue or greenish black color product shows the presence of tannins.

(c) The little quantity of the extract is treated with potassium ferric cyanide and ammonia solution. A deep red color indicates the presence of tannins.

(d) To the test extract, add strong potassium dichromate solution, a yellow color precipitate indicates the presence of tannins and phenolic compounds.

6. Test for Flavonoids

(a) The drug in alcoholic and aqueous solution with few ml of ammonia is seen in U.V. and visible light; formation of fluorescence indicates the presence of flavonoids.

(b) Little quantity of extract is treated with amyl alcohol, sodium acetate and ferric chloride. A yellow color solution formed, disappears on addition of an acid indicates the presence of flavonoids.

(c) Shinoda's Test: The alcoholic extract is treated with magnesium foil and concentrated HCl give intense cherry red color indicates the presence of flavonones or orange red color indicates the presence of flavonols.

(d) The extract is treated with sodium hydroxide; formation of yellow color indicates the presence of flavones.

(e) The extract is treated with concentrated H_2SO_4, formation of yellow or orange color indicates flavones.

(f) The alcoholic and aqueous extract is treated with 10% sodium chloride; formation of yellow color indicates the presence of coumarins.

7. Test for Steroids

(a) **Libermann-Burchard test:** 1gm of the test substance was dissolved in a few drops of chloroform, 3ml of acetic anhydride, 3ml of glacial acetic acid were added, warmed and cooled under the tap and drops of concentrated sulphuric acid were added along the sides of the test tube. Appearance of bluish-green color shows the presence of sterols.

(b) **Salkowski test:** Dissolve the extract in chloroform and add equal volume of conc. H_2SO_4. Formation of bluish red to cherry color in chloroform layer and green fluorescence in the acid layer represents the steroidal components in the tested extract.

8. Test for Proteins and Amino Acids

(a) **Biuret test:** Add 1ml of 40% sodium hydroxide solution and 2 drops of 1% CuSO4 solution till a blue color is produced, and then add to the 1ml of the extract. Formation of pinkish or purple violet color indicates the presence of proteins.

(b) **Ninhydrin test:** Add two drops of freshly prepared 0.2% ninhydrin reagent (0.1% solution in n-butanol) to the small quantity of extract solution and heat. Development of blue color reveals the presence of proteins, peptides or amino acids.

(c) **Xanthoproteic test:** To 1ml of the extract, add 1ml of concentrated nitric acid. A white precipitate is formed, it is boiled and cooled. Then 20% of sodium hydroxide or ammonia is added. Orange color indicates the presence of aromatic amino acids.

(d) **Millon's test:** 1ml of test solution is made acidify with sulphuric acid and add Millon's reagent and boil this solution. A yellow precipitate is formed indicates the presence of protein.

9. Test for Triterpenoids

(a) **Noller's test:** Dissolve two or three granules of tin metal in 2ml thionyl chloride solution. Then add 1ml of the extract into test tube and warm, the formation of pink color indicates the presence of triterpenoids.

10. Test for Fixed Oils and Fats

(a) **Spot test:** Press a small quantity of extracts between the filter paper. Oil stains on paper indicates the presence of fixed oils.

(b) **Saponification test:** To 1ml of the extract add few drops of 0.5 N alcoholic Potassium hydroxide along with a drop of phenolphthalein. Heat the mixture on a water bath for 1-2 hours. The formation of soap or partial neutralization of alkali indicates the presence of fixed oils and fats.

11. Test for Gums and Mucilage

(a) Add about 10ml of aqueous extract slowly to 25ml of absolute alcohol with constant stirring. Filter the precipitate and dry in air. Examine the precipitate for its swelling properties and for the presence of carbohydrates.

12. Test for Lignins

(a) With alcoholic solution of phloroglucinol and hydrochloric acid, appearance of red color shows the presence of lignins.

The constituents present in different extracts of powdered leaves of *Cardiospermum helicacabum, Linn.* are summarized in (Table No.5).

TABLE NO. 5

QUALITATIVE PHYTOCHEMICAL ANALYSIS OF ALCOHOLIC AND AQUEOUS EXTRACTS OF *CARDIOSPERMUM HALICACABUM, LINN.*

Plant constituents	**Alcoholic extract**	**Aqueous extract**
Alkaloids	-	-
Saponins	+	+
Glycosides	-	+
Carbohydrates	+	+
Tannins and Phenolic Compounds	-	-
Flavonoids	+	+
Steroids	+	+
Proteins and Amino acids	+	+
Fixed Oils and Fats	-	-
Triterpenoids	-	-
Gum and Mucilage	-	-
Lignins	+	+

(+) : Present

(-) : Absent

5.2.5 CHROMATOGRAPHY

Chromatography is essentially a group of techniques for the separation of the compounds of mixtures by their continuous distribution between two phases, one of which is moving past the other. The main principle of the separation may be either partition or adsorption. The extract was subjected to Thin Layer Chromatographic studies for the separation and identification of their components.[50,53,55]

Thin layer chromatography (TLC) [57, 58]

In 1958, Stahl demonstrated application of TLC in analysis, a method based on adsorption chromatography. It is at present an important analytical tool for qualitative and quantitative analysis of a number of natural products, for separation, identification and estimation of different components.

The principle of separation is adsorption. One or more compounds are spotted on a thin layer of adsorbent coated on a chromatographic plate. The mobile phase solvent flows through because of capillary action (against gravitational force). The component moves according to their affinity towards the stationary phase, the component with higher affinity towards the stationary phase travels slower. The component with lesser affinity towards the stationary phase travels faster. Thus the components are separated. The information provided by a finished chromatography includes the “migrating behavior” of the separated substances.[59]

It is given in the form of the Rf value (relative to front)

Rf = Distance traveled by solute / Distance traveled by solvent

The Rf value must be in the range of 0.01-1.

TLC can be exploited in the investigation and cultivation of medicinal plants. It is possible to run many samples of extracts from different chemical races. Simultaneously with authentic standard and high performance individuals can be recognized, selected and bred. This technique is also valuable for discovery so called chemical races.

Thin layer chromatography is an important analytical tool in the separation, identification and estimation of different components. Here, when we spot a mixture of components on a TLC plates, the compounds, which are readily soluble but not strongly adsorbed moves up along with the solvent and those not so soluble but more strongly adsorbed move up less readily leading to separation of compounds.

Steps involved in TLC

(1) Plate preparation with appropriate adsorbent
(2) Activation of adsorbent
(3) Sample application as spots or bands over the chromatographic plate
(4) Solvent system selection
(5) Detecting agent/ ultra violet light
(6) Qualitative/Quantitative analysis

Procedure

- Silica gel G was weighed in required quantity.
- Homogenous slurry was made with sufficient distilled water.
- Then the slurry was poured into TLC glass plates by spreading technique and the uniform silica gel layer was adjusted to 0.25 mm thickness.
- The coated plates were allowed to dry in air and activated by heating in hot air oven at 100-105℃ for 1 hour and then used for TLC.
- The extracts were prepared with the respective solvent like ethanol and distilled water are made up to 10ml in different test tubes.
- Then with the help of capillary tube extracts was spotted on TLC plate, which was developed in TLC chamber, previously saturated with different solvent systems.
- By trail and error method, ethanol extracts showed isolation and resolution of spots with following solvent systems:

1) Chloroform : Methanol (70:30)

The different spots developed in each solvent system were identified under UV light (λ= 254 nm) and the Rf value were correspondingly calculated and tabulated (Table no.6)

TABLE NO. 6

TLC OF AQUEOUS AND ALCOHOLIC EXTRACTS OF LEAVES OF *CARDIOSPERMUM HELICACABUM, LINN*.

Sr. No.	**Extracts**	**Solvent Systems**	**No. of spots**	**Rf Values**
1.	Alcoholic Extract	*Chloroform:* Methanol(70:30)	02	0.93 0.71
2.	Aqueous Extract	*Chloroform: Methanol (70:30)*	01	0.85

5.2.6 SPECTRAL STUDIES

UV absorption spectroscopy [59,60,61,62]

The measurement of absorption of ultraviolet and visible radiation provides a convenient for the analysis of numerous inorganic and organic species.

Instrument	-	Shimadzu UV 240
Solvent	-	Water
Wave	-	200-800 nm.
Speed	-	Fast

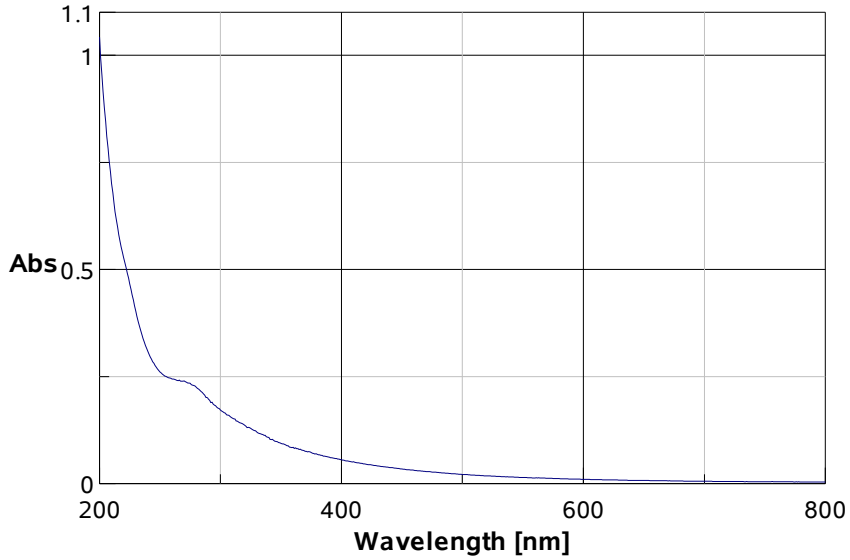
TABLE NO. 7

DATA FOR ISOLATED FRACTION FROM LEAVES EXTRACT OF *CARDIOSPERMUM HELICACABUM, LINN*.

Sample No.	Max(nm)	Absorbance
01	270	0.53215

FIG.NO. 9 UV SPECTRA OF *CARDIOSPRMUM HELICACABUM, LINN.*

λ max in water 270nm

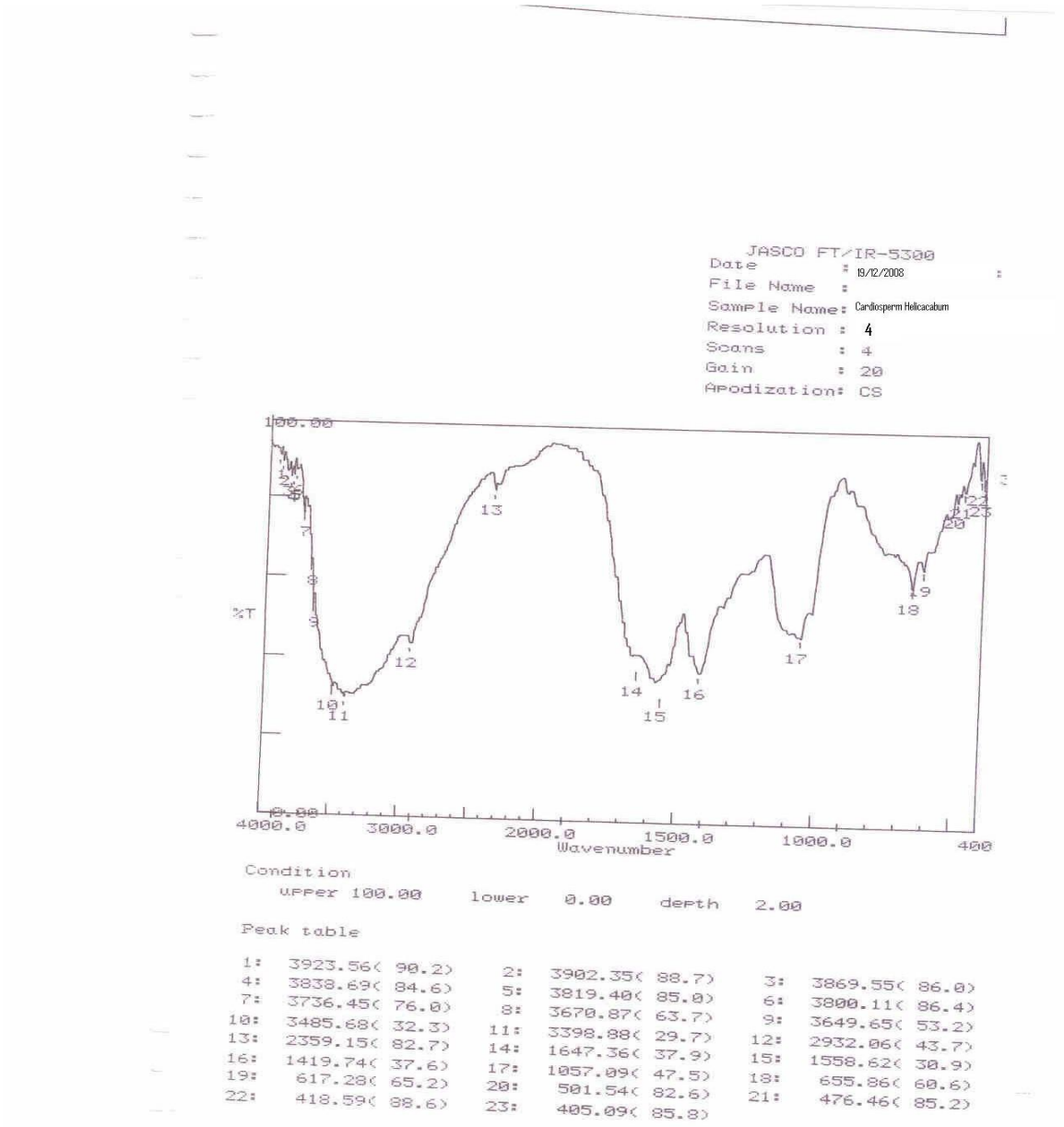


FT-IR SPECTROSCOPY [59,60,61,62]

Fourier Transform Infra-Red spectrum of the fractions obtained from the aqueous extract of powdered leaves of *Cardiospermum helicacabum, Linn.* was investigated for its characteristic functional groups. All the peaks obtained by FT-IR spectroscopy are shown in fig.

Instrument	-	JASCO FT/IR-5300
Method	-	KBr pellets method.
Wave Number	-	4000-400 cm^{-1}

FIG.NO. 10 FT-IR SPECTRA OF CARDIOSPERMUM HELICACABUM, LINN.



5.3 PHARMACOLOGICAL STUDIES

5.3.1 ACUTE ORAL TOXICITY STUDY [63, 64]

The procedure was followed by using OECD (Organization of Economic Co-operation and Development) guidelines 423(Acute toxic class method)**.**

The acute toxic class method is step wise procedure with 3 animals of a single sex per step. Depending on the mortality and / or moribund status of the animals, on the average 2-4 steps may be necessary to allow Judgment on the acute toxicity of the test substance [Fig. no. 11]. This procedure results in the use of a minimal number of animals while allowing for acceptable data-based scientific conclusion.

The method uses defined doses (5, 50, 300, 2000 mg/kg body weight) and the results allow a substance to be ranked and classified according to the Globally Harmonised system (GHS) For the classification of chemical which cause acute toxicity.

The twelve Wister rats of female sex weighing 140-180gms were used for pharmacological studies. Throughout the experimental period, the animals were provided with food and water. The alcoholic and aqueous extracts were used for the following studies.

The starting dose level of alcoholic and aqueous extracts of powered leaves of *Cardiospermum helicacabum, Linn***.** was 2000mg/kg body weight p.o. As most of the crude extracts possess LD value more than 2000mg/kg and the route of administration is oral, 2000mg/kg was used as starting dose. Dose volume was administered 0.1ml/100gms body weight to the rats, which were fasted over night with water *ad libitum*.

Food was withheld for a further 3-4 hours after administration of drug p.o.

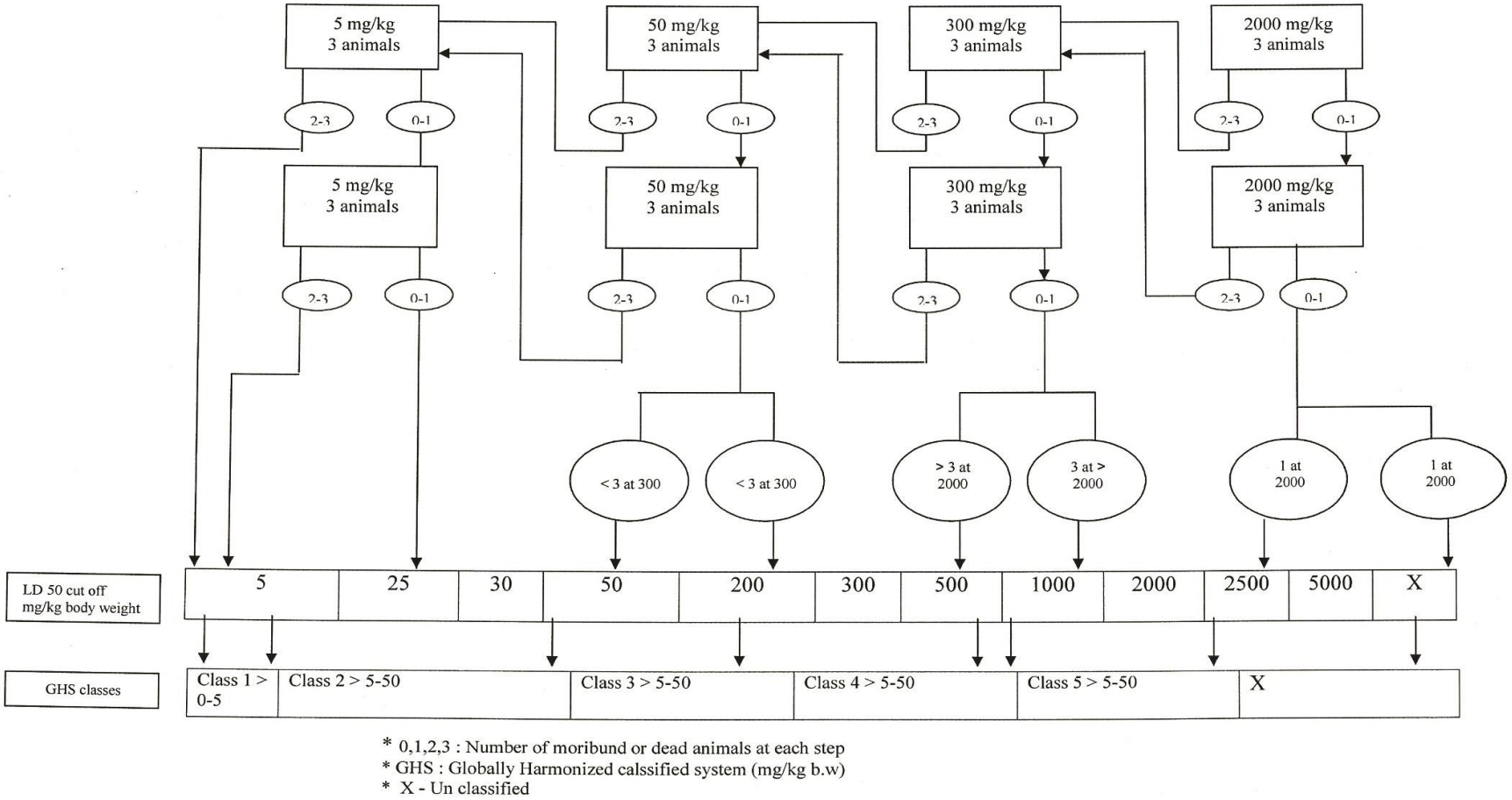
Body weights of the rats before and after termination were noted and any change in skin and fur, eyes and mucous membrane and also respiratory, circulatory, autonomic and central nervous system and somatomotor activity behavior pattern were observed. And also signs of tremors, convulsions, salvation, diarrhea, lethargy, sleep and coma were noted. The onset of toxicity and signs of toxicity was also to be noted if any. The results of toxicity studies gross behavior were reported in Table No.9

Observation

No toxicity or death was observed for these given dose levels, in the selected and treated animals. So the LD_{50} of the alcoholic and aqueous extracts as per OECD guidelines-423 is greater than 2000mg/kg (LD_{50}>200mg/kg). Hence the biological dose was fixed 200 and 400mg/kg for both the extracts.

FIG. NO. 11

Flow chart for acute toxic classmethod (OECD guidelines 423) at starting dose of 2000 mg/kg body weight/P.O



* 0,1,2,3 : Number of moribund or dead animals at each step
* GHS : Globally Harmonized calssified system (mg/kg b.w)
* X - Un classified

TABLE NO: 8 ACUTE TOXICITY CLASS METHOD (OECD GUIDELINES 423)

TABLE NO.9
BEHAVIOURAL STUDIES

Time	Alert	Passive	Grooming	Restlessness	Aggressive	Touch response	Pain response	convulsion	Gripping strength	Pin reflex	Corneal reflex	Writhing	Pupil size	Salivation	Skin colour	Lacrimatio	Respiration
1st hour	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2nd Hour	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3rd Hour	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-
4th Hour	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-
24th hour	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

- = NORMAL ++ = MODERATE EFFECT

\+ = MILD +++ = MARKED EFFECT

5.3.2 ANTI INFLAMMATORY ACTIVITY

Introduction

Inflammation was characterized, two thousand years ago by Celsius by the four Latin words rubor, calor, lumor and dolar. Inflammation has different phases; the first phase is caused by an increase of vascular permeability resulting in exudation of fluid from the blood in to the tissues and the third one by granuloma formation. Accordingly anti-inflammatory test have to be divided into those measuring acute inflammation, sub acute inflammation and chronic repair processes. In some cases the screening is directed to test compounds for local application.

The anti-inflammatory activity of the extract was screened with the help of phlogestic agent, carrrgeenan which acts as a mediator to induce inflammation in the right hind paw of albino rats.

Carrageenan induced rat paw oedema method

It is a widely used test to screen anti-inflammatory agents. It measures the ability of compounds to reduce local oedema induced in rat paw by injection of an irritant. Carrageenan which is a mucopolysaccharide obtained from Irish Sea mass *chondrous cripus*. Most anti-inflammatory agents suppress this type of oedema.[65]

It was known that carrageenan induced rat paw oedema was caused by release of histamine, serotonin in first stage and prostaglandins in the second stage. This assay is a classical in vivo method used to detect cyclo-oxygenase inhibiting activity.

The apparatus used for the determination of anti-inflammatory activity by carrageenan induced rat paw oedema method was *UGO BASINE Plethysmometer-7150.*

It consisted of two transparent parallel interconnected glass cells, one of which contained within it two platinum electrodes which were connected to the digital electronic reading device while the other call was marked at upper end which is used for dipping the rat foot. The reading device gives electronic display by which the volume of paw can be read in ml.

Reservoir containing the displacement fluid was connected to the interconnected cell and was kept at slightly higher level. The liquid used for displacement was a solution of Sodium chloride (0.04 - 0.05% w/v).

Plethysmometer worked on the principle that the paw displaced an equivalent volume of fluid, which results in a change in potential between the electrodes, which was displayed electronically on the reading device.[67,68]

Material required

Instrument	: Plethysmograph
Animal used	: Albino rats 155-200 gms.
Standard solution	: Indomethacin (100mg/kg)
Control solvent	: Carrageenan
Test solution	: Aqueous and alcoholic extract (200mg/kg.)

Methodology

Albino rats of either sex weighing 150-200 gms were divided into four groups of six animals each. All the animals were fasted over night with water and grouped as mentioned below. The dose of the drug administered to the different groups were as follows

Group I : The animals received 0.3% CMC (5ml/kg p.o.) and served as control.

Group II : The animals received aqueous extract. (200 mg/kg p.o.) suspended in 0.3% CMC.

Group III : The animals received alcoholic extract. (200 mg/kg p.o.) suspended in 0.3% CMC.

Group IV : The animals received reference standard as Indomethacin (100mg/kg p.o.) suspended in 0.3% CMC.

After one hour of the administration of the drugs, dose of 0.1ml of 1% w/v carrageenan solution in normal saline was injected in the sub planter region of the left hind paw of the rat and right hind paw served as the control. The paw volumes of the rats were measured in the digital Plethysmograph at a time interval of 30, 60, 90, 120, 150 and 180 minutes after oral drug treatment.

The degree of oedema formation at the hind paw volume was measured by plethysmographical method. The volume displacement has been expressed as percentage activity in reducing oedema and was determined by following formula.

$$\% \text{ Inhibition} = [1 - Dt/Dc] \times 100$$

Where, Dt = mean relative change in paw volume in test group.

Dc = mean relative change in paw volume

TABLE NO: 10

EFFECT OF *CARDIOSPERMUM HELICACABUM, LINN.* IN CARRAGEENAN INDUCED PAW OEDEMA IN WISTER ALBINO MALE RATS

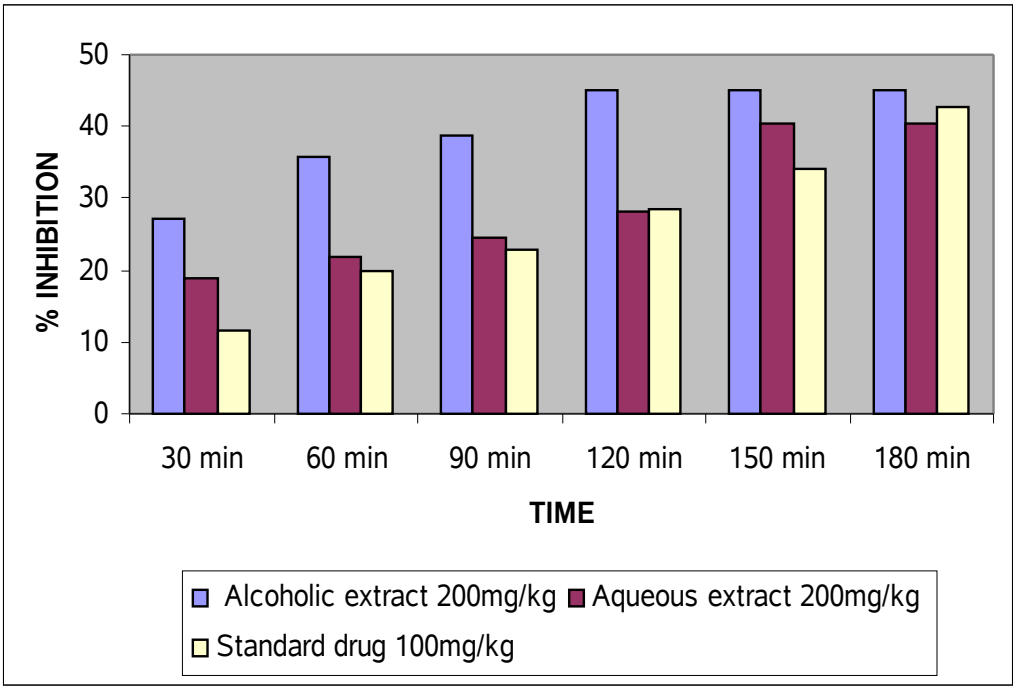
Values are expressed as mean ± s.d. (n=6).

The percentage activity is given in parentheses.

* = $P < 0.05$

** = $P < 0.01$

FIG.NO.12 GRAPHICAL REPRESENTATION OF ANTI-INFLAMMATORY ACTIVITY OF *CARDIOSPERMUM HELICACABUM, LINN.*



5.3.3 DIURETIC ACTIVITY

Experimental design

Animal used	: Wister albino rats of either sex.
No. of animals per group	: 6
Average weight	: 150-180 gms.
Route of Administration	: Oral.

Methodology

Wister albino rats of either sex was selected and divided into 4 groups of 6 animals in each group.[66]

Group I	Received normal saline served as control.
Group II	Received Furosemide (100mg/kg)
Group III	Received Aqueous Extract. (200mg/kg)
Group IV	Received Alcoholic Extract (200mg/kg)

Diuretics are drugs that cause a net loss of sodium and water in urine. These drugs are used in the pathological condition associated with retention of sodium and water in body. (Example congestive heart failure, pulmonary oedema and hypertension). The important site with regard to renal function is the glomerular itself. Drugs which increase glomerular filtration rate produce diuretic effect.

The Lipschitz method of was employed for the assessment of diuretic activity. Four groups of male albino rats were fastened and deprived of water for 18 hours. Prior to the experiment, animals were given primary dose of normal saline 25 ml/kg orally.[69,70]

The alcoholic and aqueous extracts were dissolved in normal saline. Group I receives normal saline as control. Group II received Furosemide (100 mg/kg) as reference diuretic group III and Group IV received aqueous and alcoholic extract respectively (200 mg/kg)

The animals were placed in the metabolic cages. After 5 hours urine was collected and measured. The sodium and potassium concentration of the urine was measured by Flame Photometer. With the help of unpaired students “t” test the significance was calculated by comparing with control. The result were tabulated in table no.11

TABLE NO: 11

DIURETIC ACTIVITY OF EXTRACTS OF *CARDIOSPERMUM HELICACABUM, LINN.*

SR. NO.	TREATMENT	DOSE	URINE VOLUME (ml)	CONCENTRATION OF IONS (mEq/Lit)		
				Na+	K+	Cl-
01	**Control**	25ml/kg	1.7 ± 0.11	95.18 ± 0.79	95.63 ± 0.28	91.54 ± 1.3
02	**Aqueous extract**	100mg/kg	4.3 ± 0.12	128.4 ± 0.40	93.18 ± 0.25*	90.15 ± 0.86
03	**Alcoholic extract**	200mg/kg	4.1 ± 0.11	123.13 ± 0.28	93.95 ± 0.19*	91.43 ± 0.9
04	**Standard**	200mg/kg	4.4 ± 0.14	128.9 ± 0.38	94.48 ± 0.15*	92.71 ± 1.2

Significance $p<0.001$ vs. control

- Results were expressed in Mean ± SEM (Standard Error Mean)

Significance was calculated using unpaired students “t” test by comparing the extracts and standard with Control.

FIG.NO.13 GRAPHICAL REPRESENTATION OF URINE VOLUME IN DIURETIC ACTIVITY OF AQUEOUS AND ALCOHOLIC EXTRACT OF *CARDIOSPERMUM HELICACABUM, LINN.*

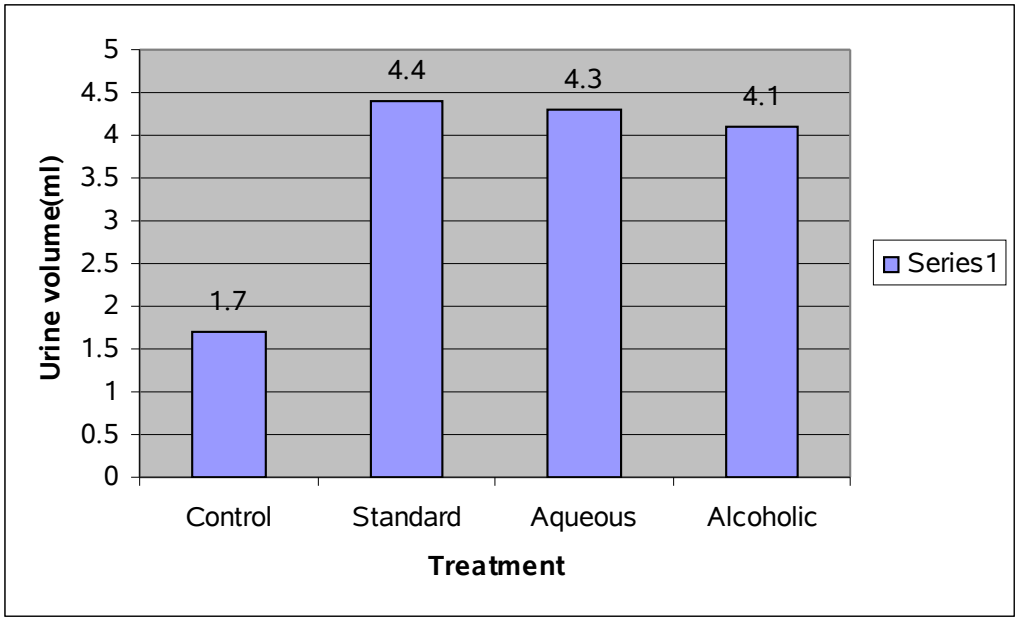
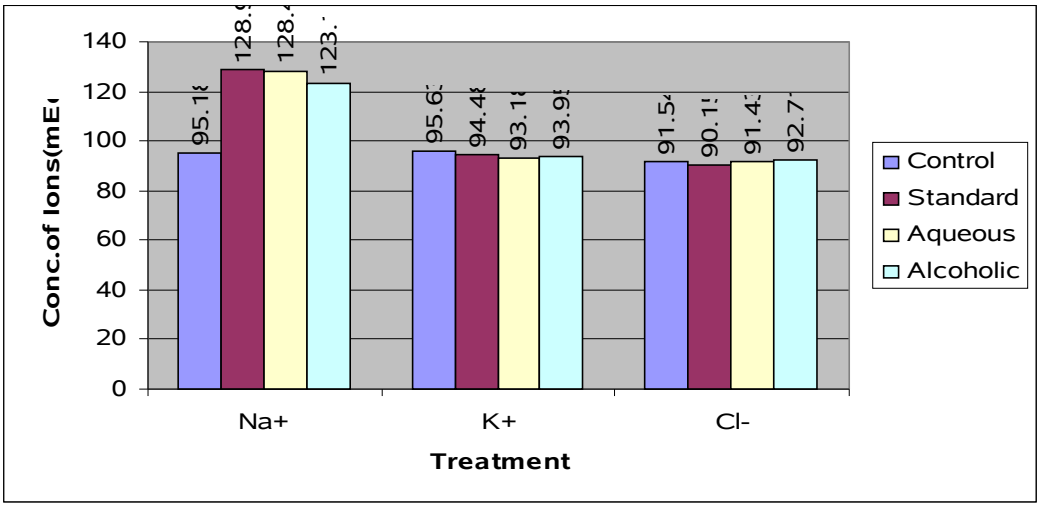


FIG.NO.14 GRAPHICAL REPRESENTATION OF CONC. OF IONS IN DIURETIC ACTIVITY OF AQUEOUS AND ALCOHOLIC EXTRACT OF *CARDIOSPERMUM HELICACABUM, LINN.*


140
120
100
80
60
40
20
0
Conc.of Ions(mE
95.1
128.
128.
123.
95.6
94.4
93.1
93.9
91.5
90.1
91.4
92.7
Na+
K+
Cl-
Treatment
Control
Standard
Aqueous
Alcoholic

6. RESULT AND DISCUSSION

The plant *Cardiospermum helicacabum, Linn*. belonging to the family *Sapindaceae* was selected for the project on the basis of ethno botanical information and easy availability.

So we can validate scientifically for folk claim its therapeutic activity. We have also undertaken its detailed pharmacognostical, phytochemical and pharmacological investigations to give it appropriate identification and rationalize it use as drug of therapeutic importance.

Pharmacognostical Studies:

The macroscopical, microscopical studies and physicochemical constants of the leaves of *Cardiospermum helicacabum, Linn.* were studied in detail and following characteristics were observed.

Microscopical studies

The lamina is 200 – 300µm thick. The abaxial and adaxial surfaces are smooth and even. The lamina is dorsiventral and mesomorphic. The adaxial epidermis cells of varying size and shape. The abaxial epidermis has narrowly cylindrical cells. The mesophyllis is differentiated in to abaxial palisade zone and abaxial spongy parenchyma zone. The palisade zone is 100-150 µm thick. It is 2 or 4 layered, compact. The spongy parenchyma consists of wide lobed 5 or 6 layers of cells which are interconnected from reticulations. The lateral veins are prominent they are collateral and have single layered, hyaline bundle sheath cells.

The marginal part of lamina is bleeont; the epidermal layers are similar to the mid part of the la mina. The palisade zone and spongy parenchyma zone continue with margin. The lateral veins are also prominent in the margin. The margin is about 150µm thick.

In cross sectional view the midrib is more or less biconvex with adaxial hump and abaxial broad conical body. It is 500µm in vertical axis. The adaxial hump is 200µm wide the abaxial part is 350µm wide. The

adaxial hump has collenchymatous group tissue and prominent epidermis with squarish cells. The abaxial midrib also has district epidermal layers of cuticle cells and large parenchymatous growed parenchyma. The vascular stored in singe and bowl shaped. It consists of a few paral files of angular, thick walled xylem elements and an abaxial zone of small clusters of phloem. The vascular strands 200µm horizontacally and 100µm/vertically.

Calcium oxalate crystals are abundant with mesophyll tissue. They are large deruses. They are diffusely distributed with spongy parenchyma. They are 20-40 µm in diameter.

The lateral veins are thin and prominent they do not form distinct vein islets. District vein- islets are seen at contains places only. The islets are small or wide they are mostly squarish in shape. The vein determinations district. They are short, thick and mostly unbranched or occasionally branched once.

Stomata occur on both surfaces on the leaf. Stomata are more outer lower side than on the upper side. The stomata are predominantly diacytic with two subsidiary cells with their common walls at right angle with long axis of the guard cells the two subsidiary cells may be equal or one of them may be much larger than the other. Some of the guard cells have elliptic aperture with other have slit like closed aperture. The epidermal cells are lobed. The anticlinal walls are thick and much wavy.

The basal (proximal) part of the petiole is Plano convex with short blant rings. Epidermal layer is thin, but district comprising of small squares cells. The outer zone of the ground tissue, especially on the adaxial and abaxial parts, the cells are collenchymatous. Remaining inner portion have this walled parenchyma cells. The (distal) upper part of the petiole has long thick rings and spindle strands are two in the proximal part and three bundles in the distal part. The bundle are lop shaped and collatrons.

The powdered leaf exhibits the following element under the microscope. Broken leaf fragment with veinislers and vein terminations are seem the vein terminations are long, wavy and thick the veins are associated with the bundle sheeth parenchyma. Fairly large druses are abundant in the powder. They occur within this walled circular parenchyma cells. Small pieces of epidermal peeling are seen with powder. Stomata of parasitic type are seen in epidermal fragments. Wavy anticlinal walls and amobokd shape of the epidermal cells are other characteristic of the powdered materials.

Physicochemical Parameters

We were investigated and reported as total ash value (8.52 %w/w), acid insoluble ash value (0.80%w/w), water soluble ash value (1.86%w/w), sulphated ash value (3.98%w/w),water soluble extractive value (9.1%w/w),and ethanol soluble extractive value (7.2%w/w), loss on drying (10.93 %w/w). The above studies enable the identification of the plant material for the future investigation and from an important aspect of the drug studies.

Phytochemical Studies

Performed by starting with purification of solvents. Then powdered leaves of *Cardiospermum helicacabun, Linn.* were subjected hot extraction with ethyl alcohol and distilled water. The yield was found to be (7.13%w/w) alcoholic extract and (8.54%w/w) of aqueous extract. The extracts were subjects to various qualitative phytochemical tests to identify the active constituents which showed presence of carbohydrates, saponins, steroids, flavonoids, proteins and amino acids and lignins are present..

Thin layer chromatographic separation of the constituents on silica gel G plates using CHCl3: Methanol (70:30) solvent as the mobile phase showed one spot at Rf value = 0.85 in aqueous extract and two spot at Rf value=0.93, 0.71 in alcoholic extract. The detected spot was more clear after spraying with anisaldehyde sulphuric acid reagent (Yellow spot)

Then U.V. Spectroscopy of the fraction were performed which showed values of sample 1-λ max =270 nm.

I.R. Spectra of Fraction

It shows absorption peak in the region 3600-3650 cm $^{-1}$at 3649 cm^{-1} which shows presence of monomeric alcohols and phenols. The absorbance band found in the region 3300-3500 cm at 3485 cm which indicates due to the presence of amines. The absorbance band found in the region 2890-3000 cm^{-1} at 2932 cm^{-1} which is due to presence of C-H stretching of methyl and ethyl group which suggests the presence of normal alkanes. The absorbance found in the region 1620-1680 cm^{-1} at 1647 cm^{-1} is due to presence of Alkenes. The absorbance found in the region of 1500-1600 cm^{-1} at 1558 cm^{-1} is due to presence of Aromatic rings. The absorbance found in the region of 1000-1300 cm^{-1} at 1057 cm^{-1} shows the presence of alcohols, ethers. The absorbance found in the region 600-1500 cm^{-1} at 655cm^{-1} is due presence of Alkanes.

Pharmacological activity

The extract showed promising anti inflammatory activity in the present study of reduction of Carrageenan induced paw oedema (Table.10). The swelling was significantly reduced on administration of 200 mg/kg doses of alcoholic and aqueous extracts to wistar male albino rats. The maximum activity 45% was observed with 200 mg/kg at $P<0.01$ level. Significant anti inflammatory effect of the drug was observed from after administration at 200 mg /kg dose level. The paw oedema was completely absent at 120 mins observation in the alcoholic and aqueous extract (200 mg/kg) and with the standard dose treatment at 180 mins.

The tested extract was shown to possess significant anti-inflammatory potency in the reduction of Carregeenan induced paw oedema in Wister male albino rats at 200 mg/kg dose level comparable to

that of the standard drug Indomethacin. The anti-inflammatory action may be contributed by the steroidal constituent of the drug.

Diuretic activity was carried out for alcoholic and aqueous extracts. Both activity shown significant diuretic effect on rats. Aqueous extract showed more diuretic effect than that of alcoholic extract .The results were shown in Table No.11

7.CONCLUSION

The leaves of *Cardiospermum helicacabum, Linn.* belonging to family *Sapindaceae* has been studied to compare and give detailed reports on pharmacognostical, phytochemical and pharmacological studies.

The pharmacognostical studies made on the leaves of *Cardiospermum helicacabum,Linn.* like macroscopical and microscopical characters, powder microscopy, physico-chemical constants like ash values, extractive values, loss on drying, foaming index and fluorescence analysis gave valuable information. This will help correct identification of this plant for future references.

Phytochemical screening of the prepared extract was done by qualitative chemical analysis and it indicated the presence of saponins, steroids, flavonoids, carbohydrate, proteins and amino acids and lignins. Thin layer chromatography was also performed to confirm the presence of steroidal saponins.

The acute oral toxicity studies were performed as per OECD guidelines-423, fixed dose procedure, showed alcoholic and aqueous extracts of leaves of *Cardiospermum helicacabum, Linn.* up to 2000mg/kg are non toxic and safe.

Anti inflammatory action of the alcoholic and aqueous extract was evaluated with carrageenan induced paw oedema technique in albino rats (6in a group) at 200mg/kg dose levels in 0.3 % CMC. Indomethacin was used as the standard anti inflammatory drug for a separate group of animals while the control received only the vehicle orally.

The oedema induced by carrageenan administration could be effectively reduced by the drug treatment and significant reduction was shown by 200 mg/kg dose of the alcoholic and aqueous extract which was comparable to that of the standard drug Indomethacin.

Diuretic activity was carried out for alcoholic and aqueous extracts. Aqueous extract showed more diuretic effect than that of alcoholic extract.

Although the present pharmacological work shows significant anti inflammatory and diuretic effect which further pharmacological investigations are also needed to elucidate the mechanism of the observed effect. Further isolation of the compounds responsible for desired effect was also needed.

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LIST OF ABBREVIATIONS

T. S.	: Transverse Section
TLC	: Thin Layer Chromatography
UV	: Ultra-violet
FT-IR	: Fourier Transform Infra Red
OECD	: Organization for Economic Co-operation and Development
FDP	: Fixed Dose Procedure
LD_{50}	: Lethal Dose 50
MEq	: Mole Equivalent
SEM	: Standard Errors of Mean
SE	: Standard Error
w/w	: Weight by Weight
w/v	: Weight by Volume

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